EPIGENOMIC ANALYSIS OF POSTTRAUMATIC STRESS DISORDER IN FEMALE RAPE SURVIVORS IN SOUTH AFRICA

JANI NÖTHLING

Dissertation presented for the Degree of Doctor of Philosophy in the Department of Psychiatry, Faculty of Medicine and Health Sciences at Stellenbosch University

Supervisor: Prof SMJ Hemmings

Co-supervisors: Prof S Seedat; Prof N Abrahams

March 2021

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), the reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes 1 original paper published in a peer-reviewed journal and 3 unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself.

Jani Nöthling
March 2021

SUMMARY

Compared to other trauma types, rape is associated with a high risk of developing posttraumatic stress disorder (PTSD). Women are at increased risk of developing PTSD compared to men and are also more frequently victims of sexual assault. PTSD is a complex, multifactorial disorder and an array of demographic, trauma-related, psychological and genetic putative risk and protective factors mediate or contribute to the development and course of the disorder.

Few studies have comprehensively investigated demographic and psychological risk and protective factors for PTSD in a longitudinal prospective design, especially beyond the 3-month post-rape period and in low- to medium-income countries. There are currently no known epigenome-wide association studies (EWASs) investigating differential methylation in relation to PTSD in (1) an African population and (2) a sample of rape-exposed women exclusively. There are also no known studies investigating longitudinal change in the hypothalamic-pituitary-adrenal (HPA) axis associated candidate gene FK506 binding protein *(FKBP5)* in relation to PTSD in a sample of rape-exposed women exclusively.

In this study we investigated the demographic, rape/assault-related, psychological, genetic *(FKBP5)* and epigenetic (epigenome-wide differential methylation) risk and protective factors associated with the development and course of PTSD symptoms over six months. Self-report measures and specimen collection was completed at baseline (within 20 day after the rape), 3-months and 6-months post-rape as part of the Rape Impact Cohort Evaluation (RICE) study. The RICE sample consisted of 852 Black African rape-exposed women, between the ages of 16 and 40 years and from a low socio-economic background.

We found that baseline demographic, rape/assault-related and psychological protective factors were not significant predictors of PTSD symptoms over time. Baseline depression and rape stigma were significant psychological risk factors for the development and course of PTSD post-rape. We also identified one intergenic CpG site (cg01700569) that was differentially methylated in relation to PTSD status at 3-months post-rape on a genome-wide level. Thirty-four differentially methylated regions were identified and included a region in the HPA-axis-associated adenylate cyclase activating polypeptide 1 *(ADCYAP1)* gene and the neuroendocrine-associated brain-specific serine/threonine-protein kinase 2 (*BRSK2)* gene. Decreased *BRSK2* and *ADCYAP1* methylation at 3-months and 6-months post-rape was associated with increased PTSD symptom scores at the same time-points. Decreased *FKBP5*

methylation was a predictor of increased PTSD symptom scores at 3-months and 6-months post-rape. High childhood trauma and the CC genotype of *FKBP5* rs1360780 resulted in decreased *FKBP5* methylation and increased PTSD scores at baseline.

The study builds on existing literature, highlighting the psychological risk factors for the development and course of PTSD in rape-exposed women. Methylation findings also build on the existing literature regarding the role of epigenetics in PTSD, although the genome-wide finding implicating differential methylation of *BRSK2* in the development of PTSD is a novel finding in human studies. The study provides evidence that both psychological and biological factors have an impact on the symptom trajectory of PTSD and that both should be considered when designing and implementing interventions for the treatment of PTSD post-rape.

OPSOMMING

Verkragting word geassosieer met ‘n hoë risiko vir die ontwikkeling van posttraumatiese stresversteuring (PTSV) in vergelyking met ander tipes trauma. Vroue loop ‘n verhoogde risiko om PTSV te ontwikkel in vergelyking met mans en is ook meer gereeld slagoffers van seksuele aanranding in vergelyking met mans. PTSV is ‘n komplekse, multifaktoriese versteuring en ‘n verskeidenheid demografiese, trauma-verwante, sielkundige en genetiese vermeende risiko en beskermende faktore bemiddel of dra by tot die ontwikkeling en verloop van die versteuring.

Min studies ondersoek die demografiese en sielkundige risiko en beskermingsfaktore vir PTSV op ‘n omvattende manier deur gebruik te maak van ‘n voornemende longitudinale ontwerp, veral buite die periode van 3 maande na die verkragting en in lae- to middel-inkomste lande. Daar is huidig geen epigenoom-wye assosiasie studies (EWAS’s) wat die verhouding tussen PTSV en differensiële metilering ondersoek in (1) ‘n Afrika-bevolking en (2) uitsluitlik vroue wat aan verkragting blootgesel was nie. Daar is ook huidig geen studies wat die longitudinale veranderinge in die hipotalamus-pituïtêre-adrenale (HPA) as geassosieerde kandidaat geen FK506-bindingsproteïen *(FKBP5)* in verhouding met PTSV in ‘n steekproef van vroue wat uitsluitlik aan verkragting blootgestel was, bestudeer nie.

In hierdie studie het ons die demografiese, verkragtings-/aanrandingsverwante, sielkundige, genetiese *(FKBP5)* en epigenetiese (epigenoom-wye differensiële metilering) risiko en beskermende faktore in verwant met die ontwikkeling en verloop van PTSV-simptome oor ses maande bestudeer. Selfrapporteringskale en versameling van monsters was by basislyn (binne 20 dae na die verkragting), 3 maande en 6 maande na die verkragting voltooi, as deel van die verkragtingsimpak kohort evaluering (VIKE) studie. Die VIKE steekproef het bestaan uit 852 Swart vroue wat aan verkragting blootgestel was, wat tussen die ouderdom van 16 en 40 jaar was en wat vanuit ‘n lae sosio-ekonomiese agtergrond gekom. het

Ons het gevind dat demografiese, verkragtings/aanrandingsverwante en sielkundige beskermende faktore op basislyn nie PTSV-simptome beduidend voorspel het nie. Depressie en verkragtingsstigma op basislyn was wel beduidende sielkundige risikofaktore vir die ontwikkeling en verloop van PTSV na verkragting. Een CpG (cg01700569) wat op ‘n genoomwye vlak differensieel gemetileer was in verhouding met PTSV-status 3 maande na die verkragting was ook geïdentifiseer. Vier en dertig differensieel gemetileerde streke was geïdentifiseer en het ‘n streek in die HPA-as geassosieerde adenilaat-siklase-aktiveerende-

polipeptied *(ADCYAP1)* geen en ‘n streek in die neuro-edokriene geassosieerde berinspesifieke-serine-drieonien-proteïen-kinase 2 *(BRSK2)* geen ingesluit.

‘n Afname in *BRSK2* en *ADCYAP1* metilering by 3 maande en 6 maande na die verkragting was geassosieer met ‘n toename in PTSV-simptoomtelling by dieselfde tydpunte. ‘n Afname in *FKBP5* metilering het ‘n toename in PTSV-simptoomtelling 3 maande en 6 maande na verkragting voorspel. Hoë kindertrauma en die CC genotipe van rs1360780 het gelei tot ‘n afname in *FKBP5* metilering en ‘n toename in PTSV-simptoomtelling by basislyn.

Die studie bou voort op die bestaande literatuur en beklemtoon die risikofaktore vir PTSV in vroue wat aan verkragting blootgestel is. Die metilering bevindinge bou ook voort op die bestaande literatuur, alhoewel die genoomwye bevinding dat differensiële metilering van *BRSK2* verband hou met die ontwikkeling van PTSV ‘n nuwe bevinding in menslike studies is. Die studie bewys dat sielkundige en biologiese faktore ‘n impak het op die verloop van PTSV-simptome en dat beide oorweeg moet word wanneer intervensies vir die behandeling van PTSV na verkragting ontwerp en geïmplimenteer word.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof Sian Hemmings, for her guidance and support during the course of this study. I am very grateful for her patience, encouragement and assistance. Thank you to my co-supervisor, Prof Soraya Seedat, for her support and for creating a research orientated environment which allows career development and allowed me to complete this study. Thank you to my other co-supervisor, Prof Naeemah Abrahams, and her team for providing the infrastructure to conduct the study and for all the long hours she spent securing funding, making sure recruitment runs smoothly and acting in the best interest of the participants. Without your support this study would not be possible.

Thank you to Shibe Mhlongo and Prof Carl Lombard for always being willing to assist with statistical analyses and for patiently answering my questions and explaining complicated statistical concepts in an easily digestible way.

Thank you to Dr Matthew Suderman for your kind assistance with the methylation analysis and for designing and providing the statistical pipelines used to analyse the methylation data.

Thank you to Dr Stefanie Malan-Muller for assisting with the systematic review, Dr Jacqueline Womersley for assisting with the *FKBP5* manuscript, Natasha Kitchin for preparing samples for shipping and to Sylvanus Toikumo who offered up his time and very patiently guided me through the methylation analysis while writing up his own dissertation. Thank you to the neuropsychiatric genetics workgroup for providing a friendly supporting environment in which to complete this project.

Thank you to Melanie Marx and Dr Eilleen Vuong who initiated their own studies, rooted in the same parent study, which allowed us to provide support to each other.

Thank you to my collogues Erine Bröcker, Sharain Suliman, Janine Roos, Patricia van Zyl and Lindi Martin for your unconditional support and always being available for a debriefing session and a word of encouragement.

A special thank you to my husband, Bertus Bekker, for all the sacrifices you made to support us in this journey. Thank you to my parents and parents-in-law, Adri and Jimmy Nöthling & Elma and Danny Bekker-Jones, for always being willing to assist with taking care

of our daughter and providing support in various ways. Thank you to our daughter, Layla Bekker, for so many welcome distractions and for blessing us with your friendly personality and love. Thank you to my furry companions, Angie and Joey, who spent many hours by my side while writing up this dissertation. Thank you to all my friends and family for always being there for me.

Lastly, thank you to the women who participated in this study and for courageously standing up against gender-based violence and for fighting for the prosecution of perpetrators. The resiliency and bravery you showed in the aftermath of a devastating trauma does not go unnoticed.

This research is supported by: (i) The South African Research Chair in PTSD hosted by Stellenbosch University, funded by the Department of Science and Technology and administered by the South African National Research Foundation, (ii) the Harry Crossley Foundation, (iii) the Hendrik Vrouwes Fund; (iv) the South African Medical Research Council (SAMRC) in terms of the SAMRC's Flagships Awards Project SAMRC-RFA-IFSP-01-2013/RAPE COHORT; (v) and the SAMRC through its Division of Research Capacity Development under the National Health Scholarship Programme from funding received from the Public Health Enhancement Fund/South African National Department of Health. The content hereof is the sole responsibility of the author and does not necessarily represent the official views of the funders.

LIST OF ABBREVIATIONS

3'UTR	3' untranslated region
450K	HumanMethylation 450K BeadChip
5'UTR	5' untranslated region
ACTH	adrenocorticotropic hormone
ADCYAP1	pituitary adenylate cyclase-activating polypeptide gene
ADCYAP1R1	pituitary adenylate cyclase activating peptide receptor 1 gene
ADHD	attention deficit hyperactivity disorder
Adj	adjusted
AHRR	human aryl hydrocarbon receptor repressor
AHS	adrenal hormone system
AIM2	interferon-inducible protein / absent in melanoma 2
ALS2	alsin rho guanine nucleotide exchange factor two
ANKRD33	ankyrin repeat domain-containing protein 33
ANXA2	annexin A2
APC5	anaphase promoting complex subunit 5
ARVs	antiretrovirals
ATP9A	ATPase phospholipid transporting 9A
AUDIT-C	Alcohol Use Disorders Identification Test – Consumption
BAGE	B melanoma antigen
BDNF	brain-derived neurotropic factor
BEGAIN	brain-enriched guanylate kinase-associated protein
BMI	body mass index
bp	base pair
BP	bipolar disorder
BRSK1	brain-specific serine/threonine-protein kinase 1
BRSK2	brain-specific serine/threonine-protein kinase 2
C21orf62	chromosome 21 open reading frame 62
C-5	fifth cytosine nucleotide
C5orf63	chromosome 5 open reading frame 63
C5orf66AS1	chromosome 5 open reading frame 66 antisense RNA
CAPS	Clinician-Administered PTSD Scale
CC2D2A	coiled-coil and C2 domain-containing protein 2A
CDH15	cadherin 15
CDRISC	Connor-Davidson Resilience Scale
CER	cerebellum
CES-D	Center for Epidemiologic Studies Depression Scale
CT	childhood trauma
ChildT	childhood trauma
CIDI-SC	Composite International Diagnostic Interview – Screening Scales
CLEC9A	C-type lectin domain family 9, member A

COL18A1	collagen type XVIII alpha 1 chain
COL1A2	collagen type I alpha 2 chain
COL9A3	collagen type IX alpha 3 chai
COMT	catechol-O-methyltransferase
CORT	cortisol
CRH	corticotrophin-releasing hormone
CRP	C-reactive protein
CRQ	Context of Rape Questionnaire
CSMD2	CUB and sushi domain-containing protein
CT	childhood trauma
CTNNA3	catenin alpha 3
CTQ-SF	Childhood Trauma Questionnaire - Short Form
CTRC	chymotrypsin C
DMPs	differentially methylated positions
DMRs	differentially methylated regions
DNHS	Detroit Neighborhood Health Study
DOCK2	dedicator of cytokinesis 2
DOK5	docking protein 5
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
DST	Department of Science and Technology
DTS	Davidson Trauma Scale
DUSP22	dual specificity phosphatase 22
EC	entorhinal cortex
EPB41L1	erythrocyte membrane protein band 4.1 like 1
EPIC	MethylationEPIC BeadChip
EREs	estrogen response elements
ESM1	endothelial cell specific molecule 1
EWAS	epigenome-wide association studies
FAM164A	family with sequence similarity 164, member A
FEZ1	fasciculation and elongation protein zeta 1
FKBP5	FK506 binding protein
FLJ46321	family with sequence similarity 75, member D1
FOXJ3	forkhead box J3
FSCN2	fascin actin-bundling protein 2
G0S2	G0/G1 switch 2
GABAA	gamma-aminobutyric acid A
GAD	generalised anxiety disorder
GCLC	glutamate-cysteine ligase catalytic subunit
GRCh37/hg19	Human Genome Build 37
GREs	glucocorticoid response elements
GRs	glucocorticoid receptors
GTP	Grady Trauma Project

GWAS	genome-wide association study
HCCA2	hepatocellular carcinoma-associated gene 2
hCG	human chorionic gonadotropin
HDAC4	histone deacetylase 4
HEXDC	hexosaminidase glycosyl hydrolase family 20 catalytic domain containing
HGS	hepatocyte growth factor-regulated tyrosine kinase substrate
HIST1H2APS2	H2A histone family, member T, pseudogene
HLA-B	human leukocyte antigen B
HM27	HumanMethylation27 BeadChip
HOOK2	hook microtubule tethering protein 2
HPA-axis	hypothalamic-pituitary-adrenal axis
HREC	Health Research Ethics Committee at Stellenbosch University
HTR3A	5-hydroxytryptamine receptor 3A
IDAT	raw probe intensity data
IL12	interleukin 12
IL12B	interleukin 12B
IL18	interleukin 18
IL-1B	interleukin 1 beta
IL-6	interleukin 6
INPP5A	inositol polyphosphate-5-phosphatase A
INTRusST	Injury and Traumatic Stress Study
IPV	intimate partner violence
KAZN	kazrin periplakin interacting protein
LC	locus coeruleus
LCN8	lipocalin 8
LEC	Life Events Checklist
LINC00599	long intergenic non-protein coding RNA 599
LINC01529	long intergenic non protein coding RNA 1529
LINC02335	long intergenic non-protein coding RNA 2335
LINC02571	long intergenic non-protein coding RNA 2571
LINE-1	Long interspersed element 1
LRRC34	leucine-rich repeat-containing protein 34
LRRC3B	leucine rich repeat containing 3B
M	mean
MAD1L1	mitotic arrest deficient 1 like 1
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
MAN2C1	mannosidase alpha class 2c member 1
MAOA	monoamine oxidase A
MAR	missing at random
MBSR	mindfulness based stress reduction
MCEE	methylmalonyl-CoA epimerase
MCMC	Markov Chain Monte Carlo

Met	methionine
MetS	metabolic syndrome
MINI	Mini International Neuropsychiatric Interview
MIR125B1	microRNA125b-1
MIR3170	microRNA3170
MIR5007	microRNA5007
MIR124	microRNA124
MRI	magnetic resonance imaging
MRS	Marine Resiliency Study
MSPSS	Multidimensional Scale of Perceived Social Support
MYT1L	myelin transcription factor 1 like
N	North
N_Shelf	North Shelf
N_Shore	North Shore
NA	not applicable
ncRNAs	non-coding ribonucleic acids
NE	norepinephrine
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NGFI-A	nerve growth factor-inducible protein A
NGS	hepatocyte growth factor-regulated tyrosine kinase substrate
NINJ2	ninjurin
NOSP	not otherwise specified
NPA	National Prosecuting Authority
NR3C1	glucocorticoid receptor nuclear receptor subfamily 3
NRF	National Research Foundation
NRG1	neuregulin1
NS	not significant
NSF	not specified
OPRM1	opioid receptor mu 1
OXT	oxytocin/neurophysin I prepropeptide
OXTR	oxytocin receptor
PACAP	pituitary adenylate cyclase activating peptide
PACR1	pituitary adenylate cyclase activating peptide receptor 1
PARD3	PAR-3 family cell polarity regulator
PARK2	parkin RBR E3 ubiquitin protein ligase
PAX8	paired box 8
PC	principal components
PCL	PTSD Checklist
PCL-C	PTSD Checklist – Civilian Version
PCR	polymerase chain reaction
PDCD61P	programmed cell death 6 interacting protein
PDL6	phospholipase D family member 6

PDS	Post-Traumatic Diagnostic Scale
PFC	prefrontal cortex
PM20D1	M20 domain-containing protein 1
PNMT	phenylethanolamine-N-methyltransferase
Pol II	RNA polymerase 2
PRISMO	Prospective Research in Stress-related Military Operations
PSS	PTSD Symptom Scale
PSS	Perceived Stress Scale
PTSD	posttraumatic stress disorder
PVN	paraventricular nucleus
RICE	Rape Impact Cohort Evaluation
RNA pol II	RNA polymerase II
RNF39	ring finger protein 39
RNF6	ring finger protein 6
rRNA	ribosomal RNA
RSS	Rape Stigma Scale
S	South
S_Shelf	South shelf
S_Shore	South shore
SAMRC	South African Medical Research Council
SCID	Structured Clinical Interview for DSM Disorders
SD	standard deviation
SDK1	sidekick cell adhesion molecule 1
SE	standard error
SKA2	spindle and kinetochore-associated protein 2
SLC16A9	solute carrier family 16 member 9
SLC38A11	solute carrier family 38 member 11
SLC39A13	solute carrier family 39 member 13
SLC6A3/DAT	solute carrier family 6, member 3
SLC6A4	serotonin transporter - solute carrier family 6, member 4
SNPs	single nucleotide polymorphisms
SNS	sympathetic nervous system
SOCA	Sexual Offences and Community Affairs unit
SORBS2	sorbin and SH3 domain-containing protein 2
SPON1	spondin 1
SPRY4	sprouty RTK signalling antagonist 4
SRIP	Self-Report Inventory for PTSD
SSRIs	selective serotonin reuptake inhibitors
STARRS	Study to Assess Risk and Resiliency in Servicemembers
STG	superior temporal gyrus
STIs	sexually transmitted infections
SVA	surrogate variable analysis
TBC1D24	TBC1 domain family member 24

TBI-VA-Boston	Traumatic Brain Injury Centre of Excellence – Veteran Affairs Boston Healthcare System
TBP	TATA binding protein
TCC	Thuthuzela Care Center
TH	tyrosine hydroxylase
THEM52B	transmembrane protein 52B
TLR8	toll-like receptor 8
TMEM51-AS1	transmembrane protein 51 antisense RNA 1
TNF-a	tumour necrosis factor alpha
TPPP	tubulin polymerization promoting protein
TPR	translocated promoter region
TRACTS	Translational Research Centre for TBI and Stress Disorders
TRMO	TRNA methyltransferase O
TSPEAR-AS2	thrombospondin type laminin G domain and EAR repeats antisense RNA 2
TSS	transcription start site
TSS1500	transcription start site 1500
TSS200	transcription start site 200
TUC338	transcribed ultra-conserved region 338
UCSC	University of California Santa Cruz
USC	University of South California
USP49	ubiquitin specific peptidase 49
Val	valine
VA-M-AA	Mid-Atlantic Mental Illness Research Education and Clinical Center PTSD Study African American cohort
VA-M-EA	Mid-Atlantic Mental Illness Research Education and Clinical Center PTSD Study European American cohort
VA-NCPTSD	Boston Veterans Affairs National Center for PTSD
VA-RR&D	Department of Veterans Affairs Rehabilitation Research and Development
VNTR	variable number of tandem repeats polymorphism
VNTR 9R	variable number tandem repeat nine repeats
Wnt	wingless-type mouse mammary tumour virus
WTC	World Trade Centre 9/11 responders study
ZDHHC11	zinc finger DHHC-type containing 11
ZDHHC14	zinc finger DHHC-type palmitoyl transferase 14
ZNF595	zinc finger protein 595
ZNF813	zinc finger protein 813

LIST OF FIGURES

CHAPTER 1 **Page**

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF TABLES

CHAPTER 4

CONTENTS

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1. INTRODUCTION

This chapter provides an overview of trauma exposure, PTSD prevalence and rape in South Africa, followed by a review of the literature related to socio-demographic, psychological and biological factors associated with PTSD. Risk and protective factors are discussed and contextualized using the biopsychosocial model as theoretical framework. Risk and protective factors are grouped into pre-trauma, peri-trauma and post-trauma factors. The literature review is followed by a brief description of the methods applied in the parent study from which this sub-study originates and the rationale, aim and objectives of this dissertation are explained.

1.1.1. Trauma exposure and PTSD prevalence in South Africa

A nationally representative study investigating the prevalence of trauma and psychiatric disorders in South Africa found that 73.8% of adult South Africans have been exposed to at least one traumatic event in their lifetime, with an average of 4.3 traumatic events experienced per participant in the sample as a whole [1,2]. The most commonly reported traumatic event was the unexpected death of a loved one (39.2%) followed by physical violence (37.6%), accidents (31.9%), witnessing death or atrocities (29.5%), a family member expose to trauma (14.1%), war events (12.2%) and sexual violence (7.6%) [1]. The prevalence of trauma exposure in South Africa is at the higher end of the range of trauma prevalence rates (41.5%-80.7%) reported in a review of nationally representative studies across 24 low- to high-income countries [3]. It is also higher than the average trauma exposure rate of 67.1% reported in the same review [3].

One of the psychiatric disorders most commonly associated with trauma exposure, especially traumas involving death, threatened death, serious injury and sexual assault, is posttraumatic stress disorder (PTSD) [4–6]. PTSD is characterized by trauma-related intrusion symptoms (upsetting memories, nightmares, flashbacks, emotional distress and physical reactivity), avoidance symptoms (thoughts/feelings, external reminders), negative alterations in cognitions and mood (inability to recall features of the trauma, negative thoughts, blame of self and others, negative affect, decreased interest in activities, feeling isolated, difficulty experiencing positive affect) and alterations in arousal and reactivity (irritability, aggression, destructive behavior, hypervigilance, heightened startle response, difficulty concentrating and sleeping) [7]. A diagnosis of PTSD is dependent on the presence of the symptoms for at least one month following the traumatic event as well as significant emotional distress and functional impairment [7]. Depression and substance use disorders also often co-occur with PTSD [8,9].

The South African 12-month and lifetime prevalence of PTSD following one of many possible traumatic events was reported as 0.6% and 2.3%, respectively [2]. Exceptionally higher prevalence rates varying between 24%-65% have been reported among survivors of rape and sexual abuse [10–12]. Rape is associated with a higher conditional risk for the development of PTSD compared to other trauma types and PTSD symptoms are often present for more than a year following the event [13]. Identifying the social, psychological and biological risk and protective factors associated with PTSD following rape is of particular importance for early intervention and prevention of PTSD, given the high risk for PTSD associated with rape and the significant emotional and functional impairment associated with PTSD [14,15].

1.1.2. Definition, prevalence and reporting of rape in South Africa

According to the South African Sexual Offences and Related Matters Amendment Act 32 of 2007, rape is defined as: "…Any person ('A') who unlawfully and intentionally commits an act of sexual penetration with a complainant ('B'), without the consent of B, is guilty of the offence of rape…sexual penetration is defined as any act which causes penetration to any extent whatsoever by - (i) the genital organs of one person into or beyond the genital organs, anus, or mouth of another person; (ii) any other part of the body of one person or, any object, including any part of the body of an animal, into or beyond the genital organs or anus of another person; or (iii) the genital organs of an animal, into or beyond the mouth of another person… it is not a valid defense for the accused person to contend that a marital or other relationship exists or existed between him or her and the complainant" [16]. According to the act, adult rape can take on many forms e.g. acquaintance rape also known as date rape (perpetrator is known to the victim), stranger rape (perpetrator not known to victim), spousal rape (perpetrator is a marital partner), compelled rape (when the perpetrator forces the victims to have sex with each other), gang rape (multiple perpetrators) and corrective rape (rape as punishment for homosexuality) [16]. The underlying theme of rape, and what defines it as a criminal offence, is that the rape survivor did not consent to the act [16].

Nationally, 41,583 cases of rape were reported to the South African Police Service in the past year (2018-2019) and the national prevalence of rape is estimated to be 2.1% [1,17]. However, rape statistics as reflected by the number of cases reported to the South African police service and are not considered indicative of the actual number of rapes occurring in a given year since underreporting of rape is very common [18,19]. It is estimated that between 76% and 97.9% of rape survivors do not report the offence to the police, which results in skewed prevalence rates and many non-convicted perpetrators [18,19]. Barriers to reporting rape include

fear of being accused of lying; shame, guilt and embarrassment; feeling pity or love towards the perpetrator; fear of retaliation; fear of the stigma associated with rape and being ostracised; fear of loss of income provided by the perpetrator; structural challenges in accessing police services, lack of trust in police and the justice system and fear of secondary victimisation [20].

Reporting rape and regaining a sense of safety, control and belief in the justice system is further complicated by the low conviction rate of perpetrators [21]. A South African regional study found that only half of reported rapes (n = 2000) resulted in arrests, 43% resulted in criminal charges, 17% went to trial and only 4% resulted in convictions [22]. The low conviction rate is, in part, due to an overburdened criminal justice system but also due to limited/incomplete evidence provided by medical, laboratory and legal personnel [21,22]. Disbelieving attitudes may further contribute to under-reporting and low conviction rates and is likely rooted in stigmatising attitudes towards rape (e.g. the victim should have done more to prevent the rape, was provoking the perpetrator, did not do enough to resist the rape etc.) [21].

A concerted effort to reduce the prevalence of rape and increase the prosecution and conviction of offenders was made by the National Prosecuting Authority (NPA) when the Sexual Offences and Community Affairs (SOCA) unit launched the Thuthuzela project and opened the first Thuthuzela Care Center (TCC) in 2006 [23,24]. TCCs offer comprehensive care to rape survivors including a medical examination, prophylactic medication, a statement taken by a police officer, psychological counselling and general non-judgemental support [23,25]. There are currently 54 TCCs across the nine provinces of South Africa and participants for this study were recruited from four of these TCCs in and around the city of Durban in KwaZulu Natal, South Africa [24]. Although TCCs have made a vital contribution to addressing the needs of rape survivors, rape prevalence and its associated diseases (e.g. HIV and other sexually transmitted infection) and disorders (e.g. PTSD, depression, anxiety, alcohol abuse) remain a significant public health concern [26,27].

1.1.3. The biopsychosocial model and the history of PTSD

Several accounts of PTSD-related symptomatology are recorded in early English literature dating back as far as 440 BC, but the disorder was first recognized by psychiatrists when physically unharmed World War I soldiers displayed extreme anxiety and fear when facing reminders of mutilated or deceased soldiers who perished in combat [28]. The number of soldiers displaying psychiatric symptoms far outweighed the number of soldiers being treated for physical injuries which indicated that the trauma associated with combat exposure had a significant impact on the health of the soldiers [29]. The term ‘shell shock’ was used to describe

the emotional shock, fear, dissociation, amnesia, muteness and catatonic behavior often observed in soldiers [28]. Although there were no know effective treatment for shell shock at the time, psychiatrists did experiment with electrotherapy and psychotherapy which indicates that it was believed that the disorder was rooted in both psychological and physical abnormalities [30].

World War II differed from World War I since military attacks were no longer targeted at soldiers only but also at civilian populations e.g. bombings, mass executions and detaining/torturing civilians in concentration camps [28]. This led to the discovery that emotional disturbances following combat exposure was not limited to soldiers since civilians also displayed distressing symptoms [29]. It was also during this time that a distinction between the symptoms associated with traumatic stress or acute stress reactions and posttraumatic stress disorder started to emerge [28]. The importance of early intervention and elements of exposure therapy was also discovered since soldiers treated at the battleground showed higher recovery rates (measured by their ability to return to battle) compared to those sent away from the battleground [31]. Some psychiatrists at the time believed that the symptoms of distress originated from cardiovascular and vasomotor abnormalities, while others followed a psychoanalytical approach in explaining the symptoms [29].

The end of World War II was followed by decades of research on the etiology, presentation and course of posttraumatic stress and many studies reported chronic posttraumatic stress symptoms in military and civilian samples [28,29]. The emotional distress observed in soldiers exposed to the Vietnam war further emphasized that the symptoms experienced by war veterans were distinct from other known psychiatric disorders [32]. PTSD was finally recognized as a distinct disorder that developed following trauma exposure when it was included as a diagnostic category in the DSM-III in 1980 [33].

In the same year that PTSD was officially recognized as a distinct disorder, the biopsychosocial model gained popularity when the American psychiatrist George Engel called for a move away from disease-orientated medicine and towards patient-orientated medicine [34]. Patient-orientated medicine would not only consider physical symptoms, but also the interaction of basic biological aspects of disease (changes in body chemistry and biomarkers of health) in conjunction with psychological aspects (learning, thoughts, mood, thinking, personality, trauma exposure, etc.) and the social/environmental/demographical context (socio-economic status, gender, social support, impact of behavior of significant others, access to healthcare etc.) of the patient [34]. Evidence linking psychological and social factors to the

diagnosis and recovery from disease lead to the mainstream adoption of the biopsychosocial model [34–37].

The biopsychosocial model, compared to the earlier biomedical model, was of particular importance in psychiatry since the field had been devalued by many physicians because the origin of disorders could not be traced back to cellular and molecular irregularities [38]. This view changed to some degree with the discovery of the first antipsychotic (chlorpromazine) and tricyclic anti-depressant (iproniazid) in the 1950's [39]. Today there is a growing body of literature linking physical abnormalities to symptoms of PTSD, mostly due to technological advances in imaging techniques and in neuropsychiatric genetics [40–42]. Although substantial evidence exists linking psychiatric symptoms to neurological functions, some remnants of the biomedical model remain today in the form of stigma surrounding psychiatric disorders [43].

In addition to biological factors associated with PTSD, various social and psychological risk and protective factors have been linked to the onset and duration of PTSD [40–42]. These factors can be grouped into pre-trauma, peri-trauma and post-trauma social, psychological and biological factors [44]. Those most consistently associated with PTSD in the context of rape and explored in this study, are presented in Figure 1 and discussed in the sections that follow.

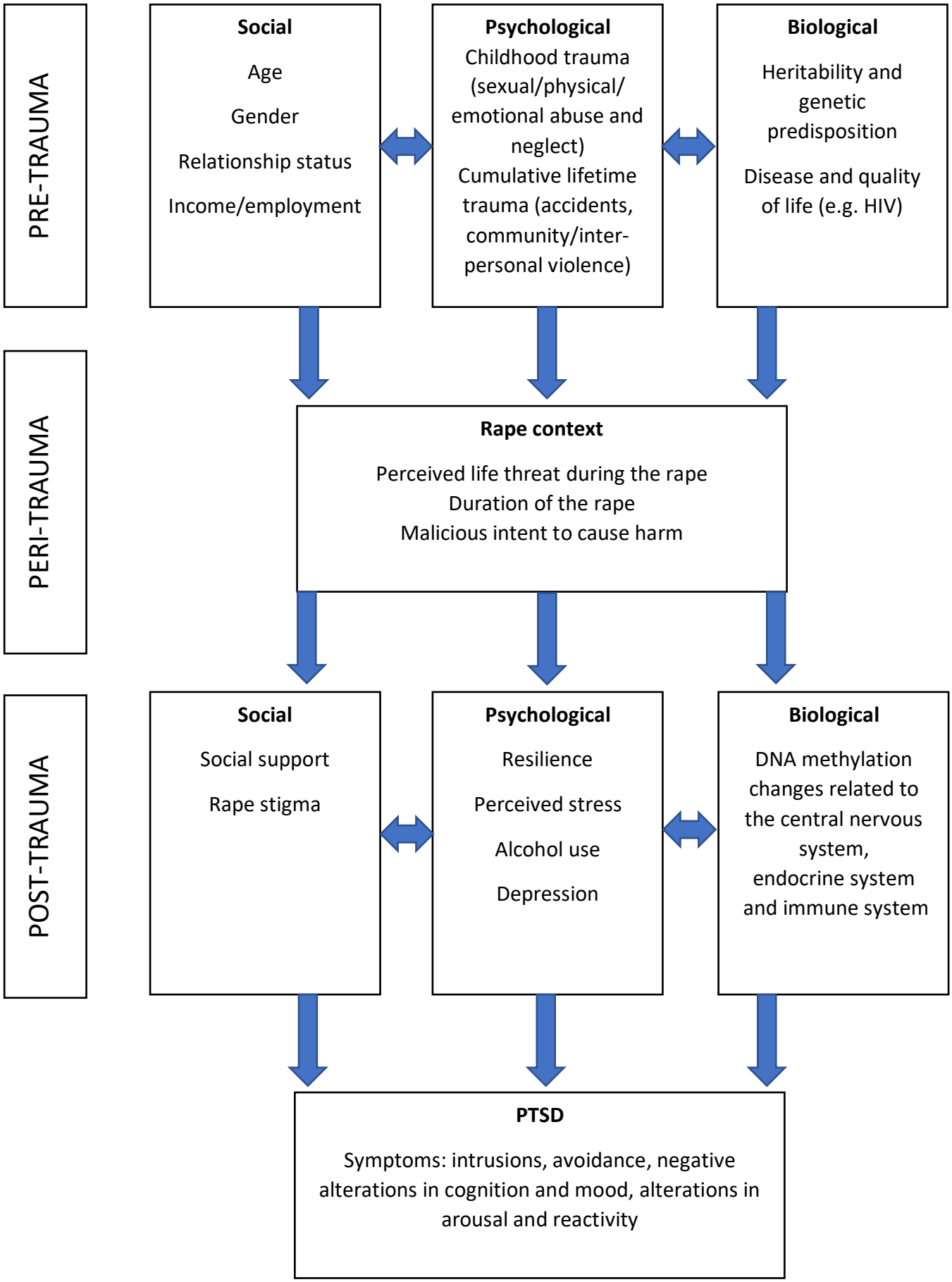


Figure 1: Biopsychosocial risk and protective factors for PTSD in the context of rape

1.2. PRE-TRAUMA FACTORS ASSOCIATED WITH PTSD

Various pre-trauma socio-demographic, psychological and biological factors have been associated with PTSD risk. These risk factors are discussed below.

1.2.1. Pre-trauma socio-demographic factors

The demographic factors most commonly investigated as risk/protective factors for the development of PTSD are age, gender, relationship status, education and income/employment [44,45]. Studies investigating large samples and systematic reviews have generally found that lower education, lower income and being single, divorced or widowed are associated with increased PTSD symptom severity [41,44–46]. The association between lower education/income and PTSD is most likely due to decreased availability of resources to cope with the trauma and an environment where trauma and violence occur more frequently [47]. Being single, divorced or widowed may decrease social support as a positive coping mechanism following trauma [45]. Findings related to age as a predictor of PTSD symptom severity are inconsistent [41,46]. Some studies report that lower age is associated with increased risk for PTSD [46] while a study comparing urban and rural samples found that older age was associated with PTSD in both the urban and rural groups [41].

By far the most consistent finding, although contested by some, is the association between female gender and PTSD [44,45]. Some studies report a two-fold increase in PTSD severity in women while others report as much as a seven-fold increase [41,44,45,48]. Whether women are more likely to develop PTSD following trauma exposure or are just more likely to disclose the trauma and seek help, remains unclear [49]. It has also been found that women are at increased risk for PTSD but only when exposed to specific trauma types such as physical and sexual assault [45]. Gender-specific characteristics of the central nervous system and the endocrine system may further confer risk for PTSD in women and are discussed in the section dedicated to post-trauma risk factors [50,51].

1.2.2. Pre-trauma psychological factors

Childhood trauma and especially multiple, chronic and repeated trauma is significantly associated with adult revictimization and PTSD [52–57]. Childhood sexual abuse has been associated with adult sexual and physical abuse and an increased likelihood to develop PTSD following revictimization in a female rape-exposed sample [58]. Childhood abuse has also been associated with more complex and severe PTSD symptoms following revictimization [59,60].

Children exposed to chronic abuse are likely to display disturbances in affective and interpersonal self-regulation [60], e.g. inability to form safe and appropriate boundaries with others, low self-esteem and more tolerance towards abuse [58,59,61–63]. Emotional development during childhood is therefore negatively affected by abuse and the consequential maladaptive coping, social and emotional skills predispose child abuse victims to adult revictimization [64,65].

Cumulative lifetime trauma may involve multiple exposures to the same trauma type or exposure to several different trauma types e.g. natural disasters, fire or explosion, transport accidents, other serious accidents, exposure to toxic substances, physical assault, assault with a weapon, sexual assault, unwanted sexual experiences, combat/war exposure, captivity, severe human suffering, sudden violent death, sudden accidental death and serious injury, harm or death [7,66]. Increased exposure to lifetime trauma has been associated with greater PTSD symptom severity and complexity in studies investigating participants exposed to various trauma types and those investigating sexual assault and rape only [61,67–69]. Cumulative lifetime trauma may result in distrust of others and a view of the world as a dangerous place [61,62,68,69]. This association me be strengthened with every additional trauma exposure and may decrease positive coping styles and increase risk for PTSD and other psychiatric disorders [62,68].

1.2.3. Pre-trauma biological factors and disease

A genetic predisposition may increase vulnerability to PTSD as well as pre-existing medical conditions interacting with biological mechanisms implicated in PTSD (e.g. hypothalamic-pituitary-adrenal axis functioning and neurocircuitry) [40,70–72]. Various diseases may also have an impact on quality of life and indirectly affect mental health [73]. Chronic diseases in particular, may increase stress through persistent fears and worries about the long-term impact of the disease, fear of dying, fear of medical procedures, long-term adverse side-effects of chronic medication use and an inability to come to terms with a diagnosis [74]. HIV is of particular concern in the context of rape given that it is sexually transmitted [75]. It is also of particular concern in the South African context given the high prevalence rate in the country [76]. Heritability, genetic variation and HIV infection as risk factors for the development of PTSD are discussed below.

1.2.3.1. Heritability of PTSD and genetic variation

A number of twin studies have investigated the heritability of PTSD using an overlapping sample of male, predominately Caucasian, North American Vietnam veterans [40]. These studies have reported a heritability rate of between 23% and 30% for PTSD and a 62.5% heritability

rate for PTSD-depression comorbidity [77–79]. Another study investigating the same Vietnam veteran sample reported a 41% heritability rate for internalizing disorders (depression, dysthymia, generalized anxiety disorder, panic disorder and PTSD) and a 69% heritability rate for externalizing disorders (antisocial personality disorder, alcohol abuse/dependence, drug abuse/dependence and PTSD) [80]. Only one study has investigated a mixed gender sample and reported a 38% heritability rate for PTSD [81]. Heritability in a North American female sample was investigated in one study and a much larger heritability rate, compared to male studies, of 71% was reported in this study [82]. The higher heritability rate may be explained by the age of participants since trauma exposure was measured when participants were between 12 and 19 years old and PTSD was measured when participants were 21.7 years old [83]. Childhood and adolescent trauma exposure are associated with an increased risk for PTSD likely due to heightened brain plasticity during these developmental periods [83,84]. Prior twin studies included older participants (30-53 years old) with trauma exposure predominately measured in adulthood [77–79]. However, this increased heritability rate in the female sample also corresponds to a prior finding indicating that women have a two to three-fold increased risk for developing PTSD following trauma exposure compared to men [50,85].

Although twin studies provide evidence of the general heritability of PTSD by comparing PTSD prevalence in monozygotic twins and dizygotic twins, they do not provide evidence for the specific genes conferring risk for PTSD [77]. Single nucleotide polymorphism (SNP) heritability studies investigate the proportion of common, genome-wide SNPs associated with PTSD in unrelated individuals [86]. The first large SNP heritability study in PTSD (N = 20 070), which comprised seven African American, nine European American, one Hispanic American and two South African samples, reported a statistically significant 29% SNP heritability rate for PTSD in female participants [87]. Heritability among male participants was 7% but this finding did not reach statistical significance [87]. They also investigated single gene variants and gene pathways as predictors of PTSD, but none of the gene variants or pathways investigated reached genome-wide significance [87]. This study was expanded upon to include data from 60 ancestrally diverse populations (n = 206 665) [88]. A SNP heritability rate ranging between 5% and 20% was observed, with this effect being driven by female participants [88]. Five SNPs, annotated to zinc finger DHHC-type palmitoyltransferase 14 *(ZDHHC14)*, parkin RBR E3 ubiquitin protein ligase *(PARK2)*, kazrin periplakin interacting protein *(KAZN)*, transmembrane protein 51 antisense RNA 1 *(TMEM51-AS1)* and zinc finger protein 813 *(ZNF813)* reached genome-wide significance in the European ethnicity subset, while five SNPs, annotated to long intergenic non-protein coding RNA 2335 *(LINC02335)*,

microRNA5007 *(MIR5007)*, transcribed ultra-conserved region 338 *(TUC338)*, long intergenic non-protein coding RNA 2571 *(LINC02571)* and human leukocyte antigen B *(HLA-B)*, reached genome-wide significance in the African ethnicity subset [88]. The two large genome-wide association studies (GWASs) expanded on the evidence implicating heritability in the etiology of PTSD specifically in females, but also indicates that several SNPs may be involved in the development of PTSD and risk alleles may differ depending on ethnicity [87].

1.2.3.2. HIV infection

Living with HIV may have an impact on various significant domains of life e.g. affordability and access to healthcare, being healthy enough to earn an income, being ostracized by the community, the prospect of having children and intimate partners, etc [89]. The fear of being infected with HIV following rape may cause significant distress to rape survivors which may add to the adverse effects of rape on mental health [90]. Living with HIV has also been associated with a significant increase in PTSD in a study investigating samples from South Africa, Nigeria, Australia, Sweden and the USA [91–93]There are several shared underlying risk factors that may explain the relationship between PTSD and HIV e.g. both PTSD and HIV are associated with: an increased prevalence of sexual abuse, physical abuse, intimate partner violence, [94] and risk behavior [91]; stigma and reduced social support [95]; neuroinflammation, altered hippocampal morphology, disruption in dopamine transmission, and altered hypothalamic-pituitary-adrenal (HPA) axis functioning [96]. Considering HIV status is therefore important when investigating the etiology and trajectory of PTSD, given the distress it may cause and the shared risk factors between the two conditions.

1.3. PERI-TRAUMA FACTORS ASSOCIATED WITH PTSD

Various trauma-related risk factors for PTSD have been investigated in general community samples and rape-exposed samples with most studies report non-significant or inconsistent findings. Trauma-related factors that have been consistently found to be associated with PTSD include perceived life threat during the exposure [44,45,48,97], length of the event [97] and malicious intent to cause harm [45,48]. Malicious intent to cause harm is also associated with chronic PTSD and less spontaneous recovery [45]. Sexual assault and rape in itself is also a risk factor for the development of PTSD compared to other trauma types such as accidents, sudden death of a loved one, physical assault, robbery and bereavement [98–100].

1.4. POST-TRAUMA FACTORS ASSOCIATED WITH PTSD

Various post-trauma psychosocial (social support, stigma, resilience, perceived stress, alcohol use, depression) and biological risk and protective factors have been associated with PTSD. Biological studies have predominately focused on the nervous system, endocrine system and immune system. These risk and protective factors are discussed below. It should be noted that some of the post-trauma risk and protective factors discussed could serve as both pre-trauma and post-trauma factors but longitudinal studies proving causality are needed to elucidate the relationship between these factors (especially epigenetic risk factors) and their role in the etiology and trajectory of PTSD following rape.

1.4.1. Post-trauma psychosocial risk and protective factors

Although women exposed to rape are at high risk of developing PTSD not all will go on to develop the disorder [10–12]. This suggests that variables other than the traumatic event are involved in the development of PTSD. Various psychosocial risk and protective factors have been associated with PTSD in multiple studies investigating various trauma types [101]. Post-trauma psychosocial risk factors include: perceived stress [102]; stigma [103]; alcohol use [8,9]; and depression [104–106]. Psychosocial protective factors include: social support [101,107,108]; and resilience [109,110]. These psychosocial risk and protective factors are discussed below.

1.4.1.1. Perceived stress

Perceived stress can be defined as a subjective appraisal of common situations in life as either stressful or not [102]. Women report significantly higher levels of perceived chronic stress and daily stressors compared to men [111]. Higher levels of perceived stress are associated with increased PTSD symptom severity and greater vulnerability for the development of PTSD [102]. Early childhood trauma and insecure attachment is also associated with a higher tendency to appraise events as stressful [112].

1.4.1.2. Rape stigma

Rape and sexual assault is often characterised by stigmatising belief and attitudes e.g. some may belief that the victim could have done more to prevent the rape or that the victim provoked the perpetrator [62,113–116]. Victim blaming, self-blaming, shame, embarrassment and decreased respect for victims are also forms of rape stigma and has consistently been found to be associated with PTSD in rape and sexual assault studies [61,114–116]. Knowing the perpetrator is

especially linked to rape stigma and victim blaming from family members and friends [62,113–116]. Less severe physical injuries has also been linked to stigmatising beliefs from health-care providers, police officials and legal representatives [59,62,69,117,118]. Not only is stigma linked to decreased mental health treatment-seeking but it is also linked to decreased physical health treatment-seeking and access to post-exposure prophylaxis for the prevention of HIV [26].

1.4.1.3. Alcohol use

A 25.3% PTSD prevalence rate has been found among treatment-seeking participants with alcohol or drug abuse/dependency [119]. Elevated PTSD symptom trajectories have also been linked to alcohol use disorders in rape and sexual assault survivors [120] and childhood trauma has been identified as a risk factor for alcohol or substance abuse/dependence in adulthood [121]. It has been hypothesized that substance abuse increases susceptibility to PTSD following trauma exposure, that individuals with comorbid PTSD and substance abuse/dependence are psychologically vulnerable for mental health disorders, and that substance abuse or dependence is a form of self-medication in the aftermath of trauma [122,123]. Sexual assault victims who receive more negative social reactions are also more likely to develop PTSD and alcohol abuse/dependence problems [115].

1.4.1.4. Depression

Depression is often diagnosed among individuals who have experienced trauma [104–106]. PTSD and depression comorbidity is common among survivors of trauma, with 36% to 51% of those diagnosed with PTSD also meeting diagnostic criteria for depression [104–106]. Individuals with both PTSD and depression experience more severe psychological symptoms [104,105], are more likely to have a history of trauma [105] and have lower levels of social support [104]. Rape, interpersonal violence and sexual violence have been identified as risk factors for comorbid PTSD and depression [59,69,101,118,124,125]. Women survivors of sexual assault are four to five times more likely to suffer from depression and anxiety [125] and women with a history of sexual assault are more likely to attempt suicide during their lifetime [126].

1.4.1.5. Social support

Lower levels of social support are often associated with higher PTSD symptom severity [101,107,108]. Social support is viewed as a stress management or coping strategy and perceived/actual social support from significant others play a significant role in reducing PTSD symptom severity [107]. Lower social support is also linked to increased victim blaming/shaming

and the use of avoidant coping strategies [127]. Women are more likely to receive negative responses from family and friends following a traumatic event compared to men [128] and negative social reactions are linked to increased PTSD symptom severity [61,128]. Social support is also important in re-establishing trust in others and viewing the world as a safe and reliable place following trauma exposure, especially following interpersonal and sexual traumas [59].

1.4.1.6. Resilience

Resilience can be defined as intrinsic, possibly modifiable, biological and psychological characteristics that protect survivors of trauma against the development of psychiatric disorders [129]. Lower levels of resilience are often associated with higher levels of PTSD symptom severity [109,110]. Protective factors against the development of PTSD have been identified and include: strong religious beliefs [130–132]; having a sense of humour [130]; having patience and tolerance [130], disclosure of trauma to others and bonding with fellow survivors [130,133]; positive coping styles [61,134]; self-efficacy, self-confidence and self-esteem [131,133,134]; parental affection and secure attachment [130,135]; and a high internal locus of control [134]. Individuals with higher levels of resilience may also avoid adverse coping mechanisms such as alcohol use and negative appraisal of the trauma and may find it easier to access social support and find meaning in the event [59,61,136,137,68,69,107,115,118,120,132,133].

1.4.2. Post-trauma biological factors

There is a growing body of evidence implicating biological processes in the aetiology and trajectory of PTSD, especially changes occurring in the nervous system, endocrine system and the immune system [42,138–140]. Epigenetics is the study of heritable phenotypic changes that do not involve modification of the genetic code [141]. In the last decade, there has also been increasing evidence suggesting that epigenetic factors potentially mediate the impact of environmental trauma exposure and gene expression resulting in altered neurological, endocrine and immune processes [138,139]. Epigenetic changes occur mainly through DNA methylation, histone modification and mechanisms involving non-coding ribonucleic acids (ncRNAs) [142]. Although evidence exists linking diseases and psychiatric disorders to histone modification and ncRNAs, DNA methylation changes remains the most widely studied epigenetic mechanism [142]. This is most likely due to: (1) the role of DNA methylation in regulating cell differentiation during embryogenesis, gametogenesis and development, but remaining relatively stable once differentiation has occurred; (2) DNA methylation patterns, although relatively stable, can change in response to changing environmental conditions and;

(3) the technological advancements made in the study and replication of epigenetic findings in recent year [138,141,143].

1.4.2.1. Brief overview of DNA methylation

DNA methylation refers to the addition of a methyl group (CH_3) predominantly to cytosine nucleotides[142]. CpG islands are of specific interest in methylation studies since: (1) more than 50% of the nucleotide sequence consists of CpG sites; (2) CpG islands are often unmethylated; and (3) CpG island are usually located in the promoter regions of genes [138,144]. Promoter regions are located close to transcription start sites (TSS) and binding of transcription factors to the TSS is essential for DNA expression [145,146]. Methylation of promoter regions is generally associated with decreased gene expression and silencing of genes [145,146]. DNA methylation and associated binding proteins may also result in histone deacetylase which condenses chromatin and inhibits transcription factors from binding to DNA and thereby further reduces expression of the gene [147]. Concordant methylation is important in regulating and differentiating cells, and also for long-term environmental adaptation and evolution, but when gene methylation profiles are discordant, they may result in increased risk for diseases such as autoimmune disorders, cancers and neurocognitive and behavioural disorders, including PTSD (see Figure 2) [148,149].

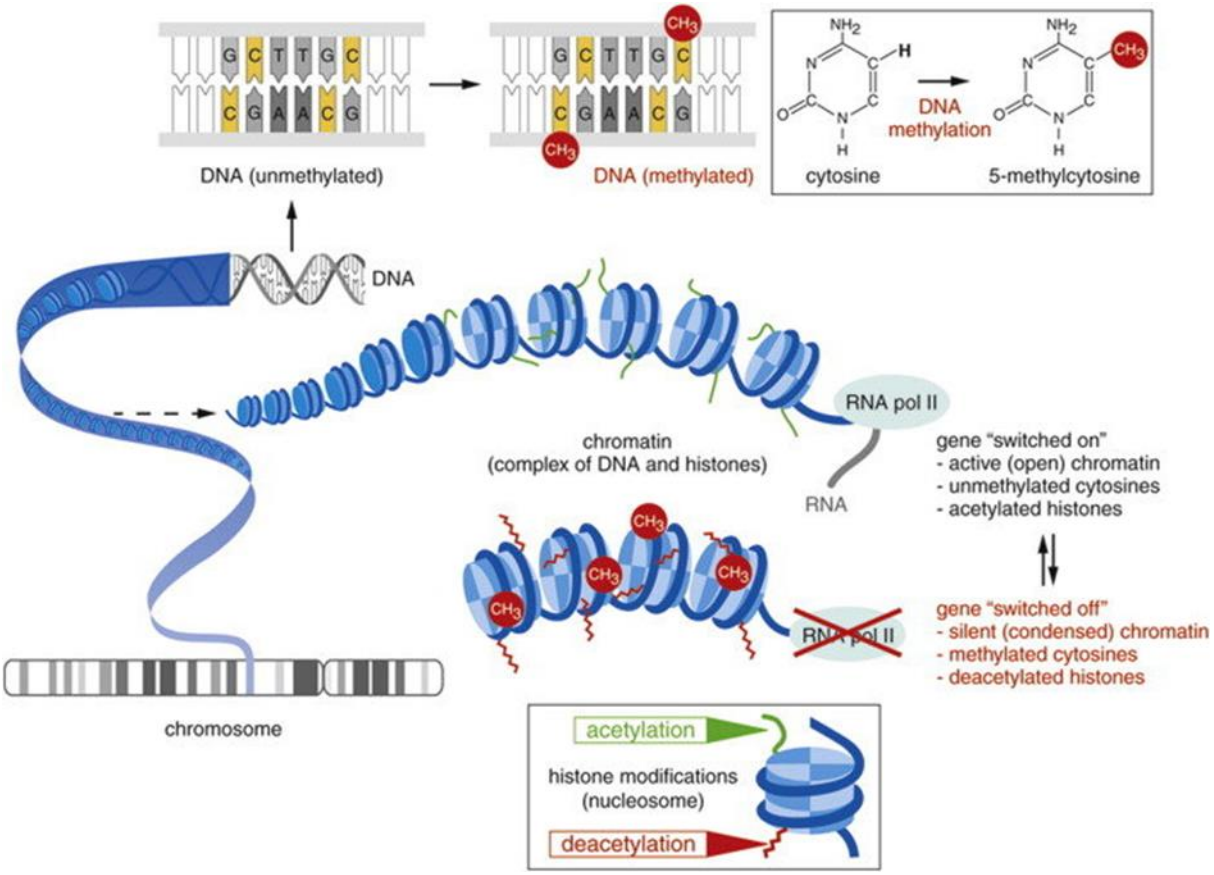


Figure 2: DNA methylation as a regulator of gene expression. Addition of a methyl group (CH_3) to CpG sites in the promoter region of a gene results in decreased expression of the gene. DNA methylation and histone deacetylase condense chromatin and further inhibits transcription of genes since RNA polymerase II (RNA pol II) is unable to bind to DNA and transcribe messenger RNA. Reprinted from "Insects as models to study epigenetic basis of disease" by K Mukherjee, RM Twyman, A Vilcinskas, 2015, *Progress in Biophysics and Molecular Biology, 118,* p. 71, Copyright 2015 by Elsevier Incorporated [147].

1.4.2.2. Methods to investigate DNA methylation

Earlier studies investigating DNA methylation in relation to PTSD mainly relied on hypothesis-driven studies investigating differential methylation at CpG sites in single genes, which were selected based on evidence linking the protein coded by the gene (e.g. cortisol, serotonin, epinephrine) to PTSD [150]. These studies relied on laboratory methods such as immunoprecipitation, bisulfite conversion and pyrosequencing [138]. Many studies investigating candidate genes reported significant associations between DNA methylation and PTSD status/severity which resulted in valuable insights into the role of methylation as a potential mediator of psychiatric symptoms (see Table 1 for details) [151]. However, CpG site-specific findings identified in this way have rarely been replicated [152,153]. Candidate gene studies also come with unavoidable limitation, e.g. they are based on *a priori* knowledge and genes not expected to be related to the biology of the disorder of interest are overlooked; the gene investigated may be relevant to PTSD but the effect may be driven by genes upstream or downstream of the gene under investigation; the selected CpG sites usually represents a small section of the gene only and are usually located in the promoter region of the gene while methylation of other regions in the gene may also contribute to the phenotype of interest; most candidate gene studies are underpowered and heterogeneity in terms of trauma exposure, socio-economic status, age, ethnicity etc. dilutes the findings since the aforementioned factors may be related to differential methylation in and of themselves [154–156].

The alternative to candidate gene designs are hypothesis-free, data-driven methods investigating methylation across the entire genome [157]. While technologies such as whole-genome bisulfite sequencing exist, it is questionable if and when large enough PTSD cohorts will exist in order to truly have adequate statistical power to apply these methods [157,158]. High-density microarray chips are considered representative of the genome and are currently the preferred method for investigating genome-wide differentially methylated genes [138]. Microarray chips can investigate close to a million sites across the genome and stringent criteria (e.g. $p < 5 \times 10^{-8}$) are used to determine genome-wide statistical significance [159]. Genome-wide studies are not confined by prior knowledge and may result in identification of novel genes and pathways involved in the aetiology of PTSD [138,158]. Secondary analyses using laboratory techniques previously employed to investigate candidate genes can further substantiate the finding resulting from genome-wide studies and may also expand the findings by investigating additional CpG sites not represented on microarray chips [138,158]. However, genome-wide studies are not without limitations and have been criticised for producing small effect sizes and non-replicable results [138]. To date, only twelve studies investigating methylation in relation to PTSD

have followed a genome-wide approach [160,161,170–173,162–169]. While most genome-wide studies have reported valuable and significant finding, few have replicated findings, and only two have followed a longitudinal design [162,163]. Similar to candidate gene studies, the discrepancies in findings is most likely due to small sample sizes and heterogeneity related to trauma exposure, PTSD measure, socio-economic status, age, ethnicity etc, within and between studies [159].

The findings of candidate gene and genome-wide studies investigating differential methylation in relation to PTSD are discussed below (for a detailed review of genome-wide studies see chapter 4). The differentially methylated genes previously identified are group into findings related to: (1) the central nervous system; (2) the endocrine system and; (3) the immune system. A summary of the methodology and finding of candidate gene studies investigating the relationship between PTSD and methylation is presented in Table 1.

Table 1: *Summary of findings in candidate gene studies investigating DNA methylation and PTSD*

Reference	PTSD measure	Gene name[1]	Design and sample size	Setting and trauma type	Ethnicity	Gender & mean age	Array and tissue type	Genomic position[2]	Finding
Ressler et al., 2011[174]	PSS	*ADCYAP1 R1*, TSS1500	Cohort, cross-sectional	Community sample with high trauma exposure (Grady Trauma Project)	Predominately African American (92.6%)	94 Males and Females NOSP. Males (36.5%) and Females (63.5%) in parent study; mean age NS.	Illumina HumanMethylation27 BeadChip; blood	Chr7:31091718	↑ Methylation at one CpG site ↑ PTSD symptom severity.
Ressler et al., 2011[174]	PSS	*ADCYAP1*, NS	Cohort, cross-sectional	Community sample with high trauma exposure (Grady Trauma Project)	Predominately African American (92.6%)	94 Males and Females NOSP. Males (36.5%) and Females (63.5%) in parent study; mean age NS.	Illumina HumanMethylation27 BeadChip; blood	Chr18:905450	Relationship between methylation at one CpG site and PTSD symptom severity not significant.
Miller at al., 2017 [175]	CAPS	*AIM2* promoter	Cohort, cross-sectional; 163 with PTSD, 123 without PTSD	Military deployed to Iraq and/or Afghanistan	Predominately Caucasian American	253 Males (88.5%), 33 Females (11.5%); 32.1 years	Illumina HumanMethylation450K BeadChip; blood	Chr1:159046973	↓ Methylation at one CpG site ↑ PTSD symptom severity.

Moser et al., 2015[176]	PCL	*BDNF* exon 4	case-control, cross-sectional; 34 with PTSD, 20 without PTSD	Women exposed to intimate partner violence and non-exposed controls	Swiss (100%)	54 Females (100%); 34.3 years	Bisulfite pyrosequencing; saliva	Chr11:27744963-27744022	No significant relationship between methylation and PTSD symptom severity.
Kim et al., 2017[177]	CAPS	*BDNF*, exon1	Case-control, cross-sectional; 126 with PTSD, 122 without PTSD	Korean Vietnam war veterans	Korean (100%)	248 Males (100%); 63 years	Bisulfite pyrosequencing; blood	Chr11:27744292-27744279	↑ Methylation across the 4 CpG sites in group with PTSD. ↑ methylation at each individual site.
Norrholm et al., 2013[178]	PSS	*COMT* promoter	Case-control, cross-sectional; 98 with PTSD, 172 without PTSD	Community sample with high trauma exposure (Grady Trauma Project)	Predominately African American	98 Males (36.3%), 172 Females (63.7%); 39 years	Illumina HumanMethylation450K BeadChip	Chr22:19950040	↑ Methylation at one CpG site in group with PTSD.
Bishop et al., 2018[179]	PCL & CAPS	*FKBP5* intron 7	MBSR treatment cohort, longitudinal; 22 with PTSD, 11 treatment responders, 11 treatment non-responders	North American war (Iraq, Gulf War, Vietnam War) veterans	Caucasian American (100%)	18 Males (81.8%), 4 Females (18.2%); 59.3 years	Bisulfite pyrosequencing; blood	Chr:35558386-35558710	↓ Methylation in MBSR non-responders, post-treatment at CpG35558513.
Yehuda et al., 2016[180]	CAPS	*FKBP5* intron 7	case-control, cross-sectional; 16 with PTSD, 16 trauma-exposed without PTSD and 8 controls.	Jewish holocaust survivors and non-exposed controls residing in North America.	Jewish (100%)	14 Males (35%), 26 Females (65%); 77.5 years	Bisulfite pyrosequencing; blood	Chr6:35558386-35558710	↑ Methylation at CpG site ‘bin3-CpG6’ in holocaust survivor group with PTSD.

Kang, Kim, Choi, So, & Kim, 2018[181]	CAPS	*FKBP5* intron 7	Case-control, cross-sectional; 123 with PTSD and 116 without PTSD	Korean Vietnam veterans	Korean (100%)	239 Males (100%)	Pyrosequencing, Blood	Chr6: 35558488 & 35558513	↑ Methylation across the 2 CpG sites in group with PTSD.
Yehuda et al., 2013[182]	CAPS	*FKBP5* exon1	PE treatment Cohort, longitudinal; 16 with PTSD, 8 treatment responders; 8 non-responders	9 Vietnam veterans and 7 veterans returning from Iraq or Afghanistan – all veterans were exposed to combat trauma	Hispanic American (37.5%) African American (37.5%) Caucasian American (25%)	14 Males (87.5%) 2 Females (12.5%); 49.6 years	Sodium bisulfite mapping; Blood	Chr6:35656916-35656633	↑ Methylation (across 38 CpG sites) ↑ PTSD symptom severity.
Schechter et al., 2017[183]	CAPS	*HTR3A*, promoter	Case-control, cross-sectional; 18 with PTSD and 17 without PTSD	Women exposed to intimate partner violence and non-exposed controls	Swiss (100%)	35 Females (100%); 34.4 years	Bisulfite pyrosequencing; saliva	Chr11:113827917- 113846077	↓ Methylation in group with PTSD at CpG2_III, ↑ Methylation in group with PTSD at CpG4_III & CpG5_III.
Bam, Yang, Zhou, Ginsberg, & Leyden, 2016[184]	CAPS	*IL12B* promoter	Case-control, cross-sectional; 30 with PTSD, 42 without PTSD	Gulf war, Iran/Afghanistan veterans and war unexposed controls	17 African American (56.7%), 11 Caucasian American (36.7%), 2 others (6.6%); details of control group NS.	27 Males (90%), 3 Females (10%); 39.6 years. Details of control group NS.	Bisulfite polymerase chain reaction	Chr5:159314783-159330473	↓ Methylation across the promoter region in group with PTSD.
Uddin, Galea, Chang, Aillo, & Wildman, 2011[185]	PCL	*MAN2C1* promoter	Case-control, cross-sectional; 23 with PTSD	Community sample (Detroit Neighborhood Health Study)	79 African American (79%), 14 Caucasian	40 Males (40%), 60 Females	Illumina HumanMethylation27 BeadChip	Chr15:75661449	↑ Methylation at one CpG site x ↑ trauma load in group with PTSD.

			and 77 without PTSD		American (14%), 7 other (7%).	(60%); 45.32 years			
Ziegler et al., 2018[186]	MINI	*MAOA*, exon1/intron1	Case-control, cross-sectional; 331 with past/current PTSD and 321 without PTSD	War exposed Bosnian civilians	Bosnian (100%)	441 Males (67.6%), 211 Females (32.4%); 49.9 years	Bisulfite direct sequencing; blood	ChrX:43515613-43515840	↑ Methylation in group with PTSD at CpG 3 & 12.
Labonte, Azoulay, Yerko, Turecki, & Brunet[187]	CAPS	*NR3C1* exon 1B and 1C	Case-control, cross-sectional; 30 with current or life-time PTSD and 16 without trauma exposure or PTSD	Community sample, PTSD cases identified index traumas as motor vehicle accidents, participation in a peacekeeping mission, assault with a weapon, physical and/or sexual abuse as a child and/or adult and other.	Canadian NOSP (100%)	23 Males (50%) and 23 Females (50%); 40.2 years	Bisulfite conversion followed by EpiTYPER mass spectrometry	Exon 1B - Chr:142784071-142784522 Exon 1C - Chr5:142783095-142783528	↓ Exon 1B methylation across 29 CpG sites in group with PTSD; ↓ methylation at CpG 2-4, 11 & 14 in group with PTSD. ↓ Exon 1C methylation across 54 CpG sites in group with PTSD; ↓ methylation at CpG 40-41 and ↑ methylation at CpG 51.
Schechter et al., 2015[188]	CAPS PCL-C	*NR3C1* exon 1F	Case-control, cross-sectional; 28 with PTSD 17 without PTSD	Community members and treatment seeking women exposed to intimate partner violence	45 French Swiss (100%)	45 Females (100%); 35.4 years	Bisulfite conversion and pyrosequencing; saliva	Chr5:42783531-142783639-	↓ Mean methylation across 13 CpG sites ↑ PTSD symptom severity. No site-specific findings.

Schur et al., 2017[189]	SRIP	*NR3C1* exon 1F	Cohort, longitudinal; 92 veterans with combat exposure	Veterans deployed to Afghanistan (measures completed pre- and post-deployment)	Dutch European NOSP (100%)	92 Males (100%); 27.5 years	Bisulfite pyrosequencing; blood	Chr5: 142783936-142783528	Not significant across 52 CpG sites. No site-specific finding.
Vukojevic et al., 2014[190]	PDS	*NR3C1* exon 1F	Case-control, cross-sectional; 93 with lifetime PTSD 59 without lifetime PTSD diagnosis	Rwandan genocide refugees located in Uganda	African Rwandan (100%)	83 Males (54.6%), 69 Females (45.4%); 35 years	Bisulfite pyrosequencing; saliva	Chr5:142783566-142783639	↓ Methylation at CpG3 ↑ PTSD symptom severity.
Yehuda et al., 2013[182]	CAPS	*NR3C1* exon 1F	PE treatment Cohort, longitudinal, 16 with PTSD, 8 treatment responders; 8 non-responders	9 Vietnam veterans and 7 veterans returning from Iraq or Afghanistan – all veterans were exposed to combat trauma	Hispanic American (37.5%) African American (37.5%) Caucasian American (25%)	14 Males (87.5%) 2 Females (12.5%); 49.6 years	Sodium bisulfite mapping; Blood	Chr5:142783607-142783883	↑ Methylation (across 39 CpG sites) ↓ PTSD symptom severity.
Mcnerney et al., 2018[191]	CAPS	*NR3C1* exon 1F	Case-control; cross-sectional 61 with PTSD 61 without PTSD	Veterans with combat exposure	Hispanic American (40.2%) Caucasian America (31.8%) African American (26.2%) Other (1.7%)	122 Males (100%); 33.6 years	Sodium bisulfite mapping; blood	Chr5:142783912-142783607	↓ Methylation in group with PTSD across 39 CpG sites. ↓ Methylation in group with PTSD at CpG23 & 39.
Mcnerney et al., 2018[192]	PCL	*NR3C1* exon 1F	Cohort, cross-sectional; 67	Veterans previously deployed to Iraq, Afghanistan,	North American NOSP	59 Males (88.1%)	Bisulfite pyrosequencing; saliva	Chr5: 142783792-142783607	Not significant across 26 CpG sites. No site-specific findings.

			veterans with combat exposure.	Vietnam, Korea, multiple locations or other location		8 Females (11.9%); 46 years			
Nawijn et al., 2015[193]	CAPS	*OXTR*, exon3	Case-control, cross-sectional; 31 with PTSD and 36 without PTSD	Dutch police officers	Dutch (100%)	34 Males, 33 Females; 40 years	Bisulfite pyrosequencing; blood	Chr3:8809464-8809387	↑ Methylation in females with PTSD but not in males.
Sadeh et al., 2016[194]	CAPS	*SKA2* 3'UTR	Case-control, cross-sectional; 116 with PTSD, 83 without PTSD	Iran/Afghanistan veterans	200 Caucasian America (100%)	182 Males (91%), 18 Females (9%); 31.8 years	Illumina HumanMethylation450K BeadChip; Blood	Chr17:57187728	↑ Methylation at one CpG site ↑ PTSD symptom severity.
Boks et al., 2016[195]	SRIP	*SKA2* 3'UTR	Case-control, longitudinal; 32 with PTSD, 61 without PTSD	Military sample pre- to post-deployment to Afghanistan	93 Dutch (100%)	93 Males (100%); 27.5 years	Illumina HumanMethylation450K BeadChip; Blood	Chr17:57187728	↓ Methylation at one CpG site ↑ PTSD symptom severity.
Chang et al., 2012[196]	PCL	*SLC6A3* promoter	Case-control, cross-sectional; 62 with PTSD, 258 without PTSD	Community sample (Detroit Neighborhood Health Study)	Predominately African America (79%)	134 Males (41.9%), 186 Females (58.1%); 51.6 years	Illumina HumanMethylation27 BeadChip; Blood	Chr5:1446443	↓ Methylation at one CpG site ↑ PTSD symptom severity.
Koenen et al., 2011[197]	PCL	*SLC6A4* intron 1	Case-control, cross-sectional; 23 with PTSD, 77 without PTSD	Community sample (Detroit Neighborhood Health Study)	Predominately African American (79%)	40 Males (40%), 60 Females (60%); 45.3 years	Illumina HumanMethylation27 BeadChip; Blood	Chr17:28562220	↓ Methylation at one CpG site associated with ↑ lifetime trauma exposure x PTSD.

[1] Identified using the GENECODE database; [2] identified using the Human Genome 19 (HG19) build from the Genome Reference Consortium

Abbreviations:
PTSD Symptom Scale (PSS); pituitary adenylate cyclase-activating polypeptide 1 receptor 1 *(ADCYAP1R1)*; transcription start site (TSS); not otherwise specified (NOSP); not specified (NS); posttraumatic stress disorder (PTSD); pituitary adenylate cyclase-activating polypeptide 1 *(ADCYAP1)*; Clinician-Administered PTSD Scale (CAPS); absent in melanoma 2 *(AIM2)*; PTSD Checklist (PCL); brain-derived neurotropic factor *(BDNF)*; catechol-O-methyltransferase *(COMT)*; FK506 binding protein *(FKBP5)*; 5-hydroxytryptamine receptor 3A *(HTR3A)*; interleukin 12B *(IL12B)*; mannosidase, alpha class 2c member 1 *(MAN2C1)*; Mini International Neuropsychiatric Interview (MINI); monoamine oxidase A *(MAOA)*; nuclear receptor subfamily 3, group C *(NR3C1)*; Self-Report Inventory for PTSD (SRIP); Post-Traumatic Diagnostic Scale (PDS); oxytocin receptor *(OXTR)*; spindle and kinetochore-associated protein 2 *(SKA2)*; 3' Untranslated Region (3'UTR); solute carrier family 6, member 3 *(SLC6A3)*; solute carrier family 6, member 4 *(SLC6A4)*.

1.4.2.3. The central nervous system

Since many PTSD symptoms are rooted in cognitive functions (e.g. memory, learning, fear) it is likely that brain regions and neurotransmitters controlling these functions are altered in PTSD [198]. Neuro-imaging studies and successful pharmacological treatment of PTSD with pharmaceuticals targeting neurotransmission, provides further evidence implicating neurological systems in the aetiology and trajectory of PTSD [42,198]. The neurocircuitry of PTSD, evidence from neuro-imaging studies, neurotransmitters (catecholamines, serotonin and oxytocin), and differentially methylated genes associated with PTSD are discussed below.

1.4.2.3.1. Neurocircuitry and PTSD

Advancements in structural and functional magnetic resonance imaging (MRI) technology have contributed greatly to understanding the neurocircuitry of PTSD and affected brain regions in recent decades [198]. The brain regions most consistently identified as being altered in PTSD include the hippocampus, amygdala, hypothalamus and prefrontal cortex [198,199]. The hippocampus is involved in processing, interpreting, organising, categorising and contextualising memories [200]. Memories stored in the hippocampus are largely coherent and placed in sequential time-dependent order [201]. When faced with a threat, the hippocampus is supressed and the amygdala is activated [200]. The amygdala forms part of the limbic system and is involved in the processing of fearful emotions and initiating the fight-or-flight response via the hypothalamus [199]. When facing a threat, the hippocampus is not able to process and contextualise memories as it would under normal circumstances and environmental cues (including visual, auditory, olfactory, tactile and gustatory cues) previously identified as non-threatening may be recoded in memory as threatening cues [202]. This may result in ongoing activation of the amygdala and the fight-or-flight response when presented with a trauma cue, but in the absence of the actual threat [202].

Intrusive thoughts and memories of the event may resurface when the hippocampus attempts to consolidate previous non-threatening memories connected to environmental cues and the newly formed threatening memories connected to the same environmental cues [200]. Avoiding trauma cues and the resulting memories may strengthen the negative emotions association with these cues while at the same time diminishing the neutral non-threatening memories previously associated with these cues [201]. Engaging with a trauma memory, in the absence of an actual threat, may facilitate reconsolidation of the memory and may decrease the intensity of the emotional response associated with that memory [203]. This process may

eventually result in fear extinction, where the cue is no longer associated with fear and activation of the amygdala and the fight-or-flight response [200]. The prefrontal cortex (involved in higher order brain functioning) is the brain region where fear and response to fear is learned (fear conditioning) and can be unlearned (fear extinction) [198]. The prefrontal cortex is connected to the amygdala and mediates the fear response by inhibiting the fight-or-flight response when a trauma cue is present, but a real threat does not follow [198]. The prefrontal cortex and hippocampus are also closely connected, the hippocampus stores and recalls memories and the prefrontal cortex plays out the memories in the conscious mind where one is able to think, organise, plan, restructure and modify memories [42,202]. Metacognitive functions such as reality testing is also governed by the prefrontal cortex and can become impaired in PTSD e.g. a flashback or trauma memory may be relived and perceived as playing out in real-time rather than being interpreted as a past event or memory [204].

1.4.2.3.2. Neuroanatomical alteration and differential methylation in PTSD

The majority of studies investigating structural neuroanatomical differences between individuals with and without PTSD have found an association between reduced hippocampal volume and PTSD status/symptom severity [205–213]. Functional MRI studies on hippocampal activation have produced mixed results, with some showing increased activation of the hippocampus when presented with fearful stimuli, while others show decreased activation [210,213–217].

Inconsistent findings related to amygdala volume have also been reported in structural MRI studies with some studies reporting increased volume [210,213,218–221] and others reporting decreased volume related to PTSD [213,222–224] On the other hand, functional MRI studies have generally reported consistent results linking increased amygdala activity to PTSD status when presented with trauma-related stimuli [213,214,230,215,217,219,225–229]

Studies investigating structural and functional differences in the prefrontal cortex in relation to PTSD are less common and are complicated by the various sub-fields of the prefrontal cortex and their associated functions [231]. In general, PTSD has been associated with decreased volume in the prefrontal cortex [210,213,232–235] and with decreased activation when presented with fearful stimuli [210,213,240,214,218,219,229,236–239].

The mechanisms through which brain regions are affected in PTSD are not clearly understood, but it is thought that increased exposure to catecholamines and glucocorticoids may result in neuronal damage and degeneration, specifically in the hippocampus [241]. Increased methylation of brain-derived neurotropic factor *(BDNF*, chr11:27744279-27744292, exon1,

promoter region), which supports neuronal differentiation, maturation and survival, has been associated with PTSD in one study [177,242] and decreased expression of BDNF have been reported in many neurodegenerative diseases, such as Parkinson's disease, multiple sclerosis and Huntington's disease [243] as well as psychiatric disorders including PTSD [244], obsessive-compulsive disorder [245–247] and panic disorder [248,249]. *BDNF* also contains a common functional SNP (rs6265, exon 4) which has been associated with PTSD in several studies [250]. Substitution of the G allele with the A allele in rs6265 results in the conversion of the amino acid valine (Val) to methionine (Met) at the 66th amino acid position in the gene, and hence the name Val66Met as it is commonly referred to [251]. The Met66 allele is associated with decreased secretion of BDNF compared to the Val66 allele, and the Met66 allele has been found to be a risk factor for the development of PTSD [252–257]. Reduced expression of BDNF is associated with hippocampal atrophy which adversely affects hippocampal driven memory functions and fear extinction and prolongs PTSD recovery [250,258–262].

Differential methylation of several other genes associated with neuronal development growth and maintenance have also been associated with PTSD [163,164]. Epigenome-wide decreased methylation of the nerve growth factor (*NGF*; chr1:115844232, 5'UTR) [164], ninjurin (*NINJ2*; chr12:739980 and 740100, promoter associated), myelin transcription factor 1 like (*MYT1L*; chr2:1817351, body/opensea), paired box 8 (*PAX8*; chr2:113992921, TSS200/TSS1500/3'UTR/5'UTR) and ring finger protein 39 *(RNF39;* chr6:30039403, 30039432, 30039435 and 30039466, island in body) genes was found to be associated with PTSD in veteran studies [163].

NGF regulates growth and survival of sympathetic and sensory neurons and differential methylation of the gene may enhanced the sensitivity of the sympathetic nervous system (SNS) [263–265]. *NINJ2* is involved in axonal regeneration following injury and may represent neuronal compensation in brain regions affected by accelerated apoptosis [266–270]. The *NINJ2* gene has also been linked to the development of Alzheimer's disease and borderline personality disorder in previous studies [270,271].

Less is known about the function of *MYT1L*, *PAX8* and *RNF39*, although *MYT1L* and *PAX8* have been associated with central nervous system development [272,273] and *RNF39* has been associated with increases long-term neuronal potentiation in animal studies [274,275]. SNPs in *MYT1L* have been linked to the development of depression and schizophrenia [270,276] and SNPs in *PAX8* have been implicated in sleep disturbances, schizophrenia and PTSD in prior studies [171,277,278]. Differential methylation of *BDNF*, *NGF*, *NINJ2*, *MYT1L*, *PAX8* and *RNF39* potentially mediates the functional and structural neuroanatomical differences observed in

PTSD although further research (specifically neuroimaging genetics studies) is needed to provide insight into the association between differential methylation and altered brain regions [279].

Differential methylation of genes involved in providing the neuronal structure for neurotransmission may further add to altered neuronal development [198]. Epigenome-wide decreased methylation of brain-specific serine/threonine-protein kinase 1 (*BRSK1*; chr19:55813339, North shore of island in the promoter region) was associated with PTSD in a study on North American veterans [164]. *BRSK1* is highly expressed in the brain, specifically in the cerebellum, hippocampus and hypothalamus, and is involved in neuronal polarity and synaptic development [280]. *BRSK1* is thought to be involved in mediating the release of neurotransmitters into the synaptic cleft via exocytosis of synaptic vesicles [281]. Differential methylation and expression of *BRSK1* may therefore disrupt synaptic development and result in uncoordinated release of neurotransmitters which may also increase the risk for PTSD and other psychiatric disorders [198,282].

1.4.2.3.3. Brain structural and functional changes and differential methylation in PTSD

Neurotransmitters are involved in stimulating or inhibiting an action between brain regions and downstream physiological and behavioural outcomes [283]. Neurotransmitters most commonly implicated in PTSD include catecholamines (norepinephrine, epinephrine and dopamine), serotonin and oxytocin [282]. The neurological functioning of catecholamines, serotonin and oxytocin and their relation to differentially methylated genes are discussed below.

Norepinephrine and epinephrine

Norepinephrine (NE) functions both as a neurotransmitter and a hormone [282]. NE, when functioning as a neurotransmitter, is a regulator of the fight-or-flight response (see Figure 3) [199]. NE neurons originate predominantly from the locus coeruleus (LC) in the brain stem and projects to various brain regions including the amygdala, hippocampus, hypothalamus and prefrontal cortex [198]. When NE neurotransmitters stimulate the hypothalamus, the hypothalamus signals the adrenal cortex (through postganglionic fibres) to release the hormones, epinephrine and NE into the bloodstream [42]. In the bloodstream, NE and epinephrine mediate the activation of the SNS [42]. Epinephrine stimulates blood flow to the heart, muscles, brain and other vital organs [284]. It also enhances the senses, increases oxygen supply in the lungs and triggers the release of glucose and lipids to increase energy supply [284]. NE inhibits blood flow to the gastrointestinal tract and suppresses the immune system in an effort to

conserve energy use by these systems which are considered non-essential when facing an immediate threat [42,199].

In a study investigating cerebrospinal fluid and plasma NE levels in male combat veterans with PTSD compared to healthy controls it was reported that: (1) cerebrospinal fluid NE concentrations were significantly higher in the group of men with PTSD [285]. Another study investigating urinary catecholamine levels in a group of children admitted to hospital following traumatic injuries found that higher levels of epinephrine upon admission were associated with increased PTSD symptom severity at six weeks post-trauma [286]. Increased levels of NE and epinephrine may indicate enhanced activation of the stress response and, based on the aforementioned studies, may increase the risk of developing PTSD following trauma exposure [42,284–286].

Differential methylation of the genes coding two enzymes involved in the metabolism of catecholamines, monoamine oxidase A *(MAOA)* and catechol-O-methyltransferase *(COMT)*, have been linked to PTSD in prior studies [178,186]. MAOA is predominately involved in the metabolism of NE and serotonin and is found throughout the brain [42] while COMT is involved in the metabolism of all catecholamine neurotransmitters and is predominately expressed in the prefrontal cortex and hippocampus (Kaur & Singh, 2017, Almli, 2014). Increased methylation of *MAOA* (chrX: 43515613-43515840, exon1/intron1) [186] and *COMT* (chr22:19950040, promoter associated) [178] was associated with PTSD in two separate studies. Increased methylation of these genes may result in less enzyme activity, increased levels of catecholamines, increased alertness, mood instability and susceptibility to psychiatric disorders [288–290]. The relationship between *COMT* and PTSD may also be explained by the common functional rs4680 SNP, also known as Val158Met, in exon 3 of the *COMT* gene [288]. Similar to the *BDNF* Val66Met SNP, substitution of the G allele with the A allele at position 158 in the *COMT* gene results in the conversion of the amino acid valine to methionine [288,291]. Those carrying the Val/Val genotype have higher enzyme activity and increased stress resiliency [288,289] while Met/Met carriers have 35-50% less enzyme activity and lower stress resiliency [288–290].

Dopamine

While less is known about the function of dopamine in relation to PTSD, dopaminergic dysfunction has been linked to psychotic symptoms which is sometimes present in severe forms of PTSD [292]. Dopamine is both an excitatory and inhibitory neurotransmitter, and is associated with behaviour, sleep, concentration, learning, mood and immune function [287]. It is also thought to play a role in hyperarousal, irritability and hypervigilance in PTSD [42]. Studies have reported

increased urinary and plasma dopamine levels in individuals diagnosed with PTSD compared to controls [293,294]. NE is also thought to interacts with dopamine by stimulating the release of dopamine in the adrenal cortex and dopamine uptake, in turn, is increased in the prefrontal cortex when binding to NE transporter [282]. The interaction between dopamine and NE in the prefrontal cortex may adversely affect the recall and reconsolidation of trauma memories and increase the risk for PTSD [282].

One methylation study did not find a relationship between solute carrier family 6, member 3 (*SLC6A3* or *DAT*; chr5:1446443, CpG island in promoter region) gene methylation and PTSD, but they did find that individuals with the 9-repeat allele (17.7%) of a 40 base pair (bp) variable number of tandem repeats polymorphism (VNTR) in the 3'UTR region of the *SLC6A3* gene showed a two-fold increased risk for meeting PTSD criteria compared to carriers of the 10-repeat allele (75.4%) [196]. The interaction between the VNTR and methylation also significantly predicted PTSD [196]. Individuals possessing the 9-repeat allele *and* showing increased methylation were at increased risk for PTSD [196]. Those possessing the 9-repeat allele and showing increased *SLC6A3* promoter methylation may have a 'double hit' risk for developing PTSD following trauma exposure [196]. The 9- and 10-repeat alleles also vary in repeat motifs and often contain SNPs, which may, in turn, influence methylation levels if the number of cytosines differ between motifs[295,296]. Increased methylation may result in reduced expression of *SLC6A3* and increased dopamine in the synaptic cleft as well as decreased absorption of dopamine by the postsynaptic neuron, thereby increasing the risk for PTSD [293,294].

Serotonin

Serotonergic neurons originate predominantly in the raphe nuclei of the brainstem and are generally associated with regulating mood, anxiety, emotions, sleep, appetite and temperature [287]. Serotonergic neurons project to various areas of the brain including the amygdala, hypothalamus, hippocampus and prefrontal cortex [202]. While the exact mechanism of interacting with neurocircuits involved in PTSD is not yet fully understood, a potential mechanism of action may be the interaction between the raphe nucleus and the locus coeruleus [42]. Neurons projecting from the raphe nucleus to the locus coeruleus may inhibit the function of NE neurons and their signalling to the amygdala which may decrease the activation of the SNS responsible for the fight-or-flight response [42,198,202].

A significant decline in PTSD symptoms following treatment with selective serotonin reuptake inhibitors (SSRIs) provides further evidence for the role of serotonin in the aetiology and trajectory of PTSD [42,297]. SSRIs inhibits the functioning of serotonin transporter which

removes unabsorbed serotonin from the synaptic cleft and transports it back to the button of the presynaptic neuron, thereby terminating absorption of serotonin by the postsynaptic neuron [197,198]. Inhibiting serotonin reuptake will therefore result in increased absorption of serotonin by the postsynaptic neuron and potentially decreased activation of NE [197,198]. Furthermore, longitudinal studies investigating neuroimaging in relation to SSRI treatment and PTSD have also reported an increase in hippocampal volumes, increased activation of the of the left medial prefrontal cortex and decreased activation of the amygdala in those receiving SSRI treatment [42,297–299].

One methylation study also found that increased trauma exposure interacted with decreased methylation of the serotonin transporter - solute carrier family 6, member 4 (*SLC6A4*; chr17:28562220, island in promoter region) gene to predict PTSD status and PTSD symptom severity [197]. Another methylation study investigating the 5-hydroxytryptamine receptor 3A *(HTR3A)* found that women with PTSD showed decreased methylation at one CpG site (chr11:113846004, 5'UTR, promoter associated) and increased methylation at two other CpG sites (chr11:113846044 and 113846077, promoter associated) compared to women without PTSD [183]. The *HTR3A* gene has been associated with childhood trauma, bipolar disorder, attention deficit hyperactivity disorder (ADHD) and borderline personality disorder in previous studies [183,300–304]. Although strong evidence exists linking serotonin to PTSD symptoms (including hypervigilance, impulsivity and the frequency of intrusive memories), more studies are needed to delineate the role of serotonin in PTSD, especially given that fifteen different serotonin receptors have been identified to date and each receptor may exhibit unique and/or overlapping functions [305].

Oxytocin

Oxytocin functions both as a neuropeptide and a hormone, and is produced in the paraventricular nucleus and the supraoptic nucleus of the hypothalamus [306,307]. The hypothalamus contains various oxytocin receptors and projects oxytocinergic neurons to the hippocampus [307]. The release of oxytocin in the hippocampus has been shown to protect hippocampal neurons from neuronal degeneration caused by increased levels of glucocorticoids in periods of prolonged stress [306,307]. Oxytocin receptors are also found in the amygdala and the prefrontal cortex, where it may mediate the stress response [308,309]. Oxytocin is therefore considered to be involved in neurogenesis and to have anxiolytic properties [307,309].

When oxytocin is released in the bloodstream by the posterior pituitary gland, it functions as a hormone that is primarily involved in contraction of the uterus during childbirth

and in lactation [307,309]. Oxytocin has also been implicated as a regulator of social behaviour, including social recognition, trust and mother-infant bonding [306,307,309].

One study investigating oxytocin concentration in saliva samples of a group of male and female police officers with and without PTSD found that men with PTSD showed lower oxytocin concentrations compared to those without PTSD, but no significant difference in oxytocin levels was found between women with and without PTSD [310]. A functional MRI study investigating an overlapping sample of police officers (mixed gender) investigated in Koch et al. (2016) found that police officers with PTSD showed reduced amygdala reactivity in response to emotional faces after intranasal administration of oxytocin compared to the amygdala reactivity reported when a intranasal placebo was administered [311]. Another study investigated intranasal administration of oxytocin as an early intervention for the prevention of PTSD in a group of adults (mixed gender) presenting to an emergency department following trauma exposure [312]. They found that those with high baseline PTSD symptom severity receiving intranasal oxytocin treatment showed a significant decline in PTSD symptom severity at 45 days post-trauma compared to those who received the placebo [312]. A study investigating a large male military cohort did not find a significant relationship between oxytocin levels and the development of PTSD over time [313]. A recent meta-analysis investigating blood, urine, saliva and cerebrospinal fluid oxytocin concentrations also did not find a significant difference between those with and without PTSD [308].

One methylation study found that increased methylation of the oxytocin receptor (*OXTR*, chr3:8809437 and chr3:8809413, CpG island in exon 3) gene was associated with PTSD in females, but not in males [314]. While evidence suggest that oxytocin plays a significant role in neuronal pathways associated with PTSD, less is known about the role of peripheral oxytocin in relation to PTSD and the mediating role of methylation. Further evidence using gender-stratified samples are also needed given the aforementioned gender specific findings reported in studies investigating the role of oxytocin in PTSD [310,314].

1.4.2.4. The central nervous system and the endocrine system

The HPA-axis is one of the main systems involved in the regulation of the stress response in mammals [315]. It involves a coordinated effort between the central nervous system and the endocrine system which has downstream effects on nearly all vital physiological systems [316–318]. The pituitary adenylate cyclase activating peptide (PACAP) has been identified as a master regulator of the HPA-axis and PACAP receptor (PACR1) is abundantly expressed in the paraventricular nucleus (PVN) of the hypothalamus [316]. When PACAP binds to PACR1 in the

hypothalamus, it triggers the release of corticotrophin-releasing hormone (CRH) and the activation of the stress response [316,319,320]. CRH stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) which signals the release of cortisol from the adrenal cortex [316,321]. Cortisol, in turn, reduces CRH secretion in the hypothalamus and ACTH secretion in the pituitary gland which creates a negative feedback loop [321–323].

PACAP binding in the hypothalamus also signals the release of NE via the locus coeruleus to the effector organs of the Splanchnic nerve (stomach, liver, pancreas, intestines, adrenal gland) [324]. The adrenal medulla, as part of the adrenal hormone system, releases NE and epinephrine in the bloodstream to sustain the SNS response (see Figure 3) [316,325]. One methylation study found that increased methylation of the PACR1 gene called *ADCYAP1R1* (chr7:31091718, CpG island in TSS1500) was associated with increased PTSD symptom severity [174]. The CC genotype of the rs2267735 SNP in *ADCYAP1R1* has also been associated with increased PTSD symptom severity, decreased *ADCYAP1R1* mRNA expression and increased amygdala and hippocampal activity [174,326]. *ADCYAP1R1* polymorphisms and differential methylation of *ADCYAP1R1* may result in a dysregulated HPA-axis and increased systemic stress which may increase the risk for PTSD and comorbid physiological disorders such as cardiovascular disease [315,327].

Cortisol, as part of the stress response, acts mainly through glucocorticoid receptors in the hypothalamus which activates the negative feedback loop and returns the body to homeostasis following exposure to a stressor [321]. Glucocorticoid receptors are also found on various cell-types and does not only have an effect on neurons [328]. When cortisol binds to intracellular glucocorticoid receptors and other co-regulators, it is able to enter the nucleus, where it can upregulate or downregulated gene transcription [321,328]. Several methylation studies have investigated an overlapping region in the glucocorticoid receptor nuclear receptor subfamily 3 *(NR3C1)* gene (chr5:142783936-142783531, island in exon 1F promoter) [182,188–192]. The majority of the studies found that decreased methylation across several CpG sites was associated with increased PTSD symptom severity [182,188,191]. Differential methylation of *NR3C1* may contribute to the dysregulated negative feedback loop of the HPA-axis associated with PTSD and may also be involved in regulating transcription of genes containing glucocorticoid response elements (GREs).

Spindle and kinetochore-associated protein 2 (SKA2) has been implicated in glucocorticoid receptor transactivation with increased expression of *SKA2* resulting in increased glucocorticoid translocation to the nucleus [329]. Two methylation studies have investigated methylation of the *SKA2* gene at the same CpG site (chr17:57187728, 3'UTR) in

relation to PTSD with one study reporting an association between decreased *SKA2* methylation and PTSD [195] and the other reporting an association between increased *SKA2* methylation and PTSD [194]. Further studies are needed to elucidate the role of *SKA2* methylation in the development of PTSD, given the conflicting findings reported in these two studies [329].

FK506 binding protein (FKBP5) is another important functional regulator of the glucocorticoid receptor [330,331]. Intracellularly, cortisol binds to glucocorticoid receptors in the cytoplasm and enters the cell nucleus where it binds to glucocorticoid response elements located on the *FKBP5* gene (promoter region, intron 2, 5 and 7) [332,333]. Binding of glucocorticoid receptor to GREs upregulates *FKBP5* transcription [332,333]. FKBP5, in turn, binds to glucocorticoid receptors in the cytoplasm and inhibits glucocorticoid receptor translocation to the nucleus thereby creating an ultra-short intracellular negative feedback loop and increasing cortisol resistance [334,335].

The T allele of the rs1360780 SNP of the *FKBP5* gene has been linked to increased *FKBP5* transcription, both in the absence and presence of glucocorticoid receptor binding to GREs, compared to the CC genotype [336]. Increased expression most likely occurs as a result of the T risk allele and it adjacent alleles forming a TATA box which often represents the transcription start site in promoters [337]. Transcription is normally initiated when RNA polymerase 2 (RNA Pol II) and TATA binding protein (TBP) binds to the TATA sequence [333,337]. Binding of TBP causes the DNA strands to bend at the binding site and a three-dimensional chromatin loop is formed resulting in the GRE of intron 7 coming into direct contact with the GRE of intron 2 and RNA Pol II, thereby further enhancing transcription of *FKBP5* [336,337]. An attention bias towards threat stimuli, increased hippocampal activation and increased risk for the development of PTSD was also associated with the T allele in prior studies [333,338].

Methylation studies investigating *FKBP5* have reported conflicting findings, with some showing that decreased *FKBP5* methylation (chr6:35558513 and 35558710, intron 7) is associated with PTSD [179,180], while others showed that increased *FKBP5* methylation (chr6:35558488, 35558513, 35558710 intron 7 & chr6: 35656916-35656633, exon 1 promoter associated) is associated with PTSD [180,181]. The conflicting findings reported in *FKBP5* methylation studies may be explained by the differential expression of cortisol among psychiatric disorders which is further complicated by comorbidities [315,321,339].

Mood disorders, specifically major depressive disorder and bipolar disorder, have been linked to elevated cortisol levels, while anxiety disorders and trauma-related disorders (including PTSD) have been linked to reduced urinary, blood and saliva cortisol levels [339,340].

Some studies report an initial increase in circulating cortisol levels shortly after trauma exposure, followed by lasting decreased cortisol levels [341,342]. Although PTSD has been linked to reduced cortisol levels, the exact mechanisms through which lowered cortisol, and changes in glucocorticoid receptor activity, links to PTSD symptoms remains to be fully understood [321]. It has been hypothesised that: (1) HPA-axis underactivity and/or increased activation of the negative feedback loop results in decreased cortisol secretion, but this theory does not accurately reflect the hyper-aroused state associated with PTSD and other anxiety disorders; (2) endocrine glands downstream of the hypothalamus may be downregulated i.e. they may exhibit decreased sensitivity to the upstream signalling of the HPA-axis (starting in the hypothalamus) indicating that the HPA-axis is reprogrammed in response to chronic stress; and (3) intracellular metabolism of cortisol mediated by tissue specific changes in enzyme expression (needed to activate ligand binding for access to the cell nucleus and gene expression changes) may also be altered by chronic stress and HPA-axis activity [321,328,343].

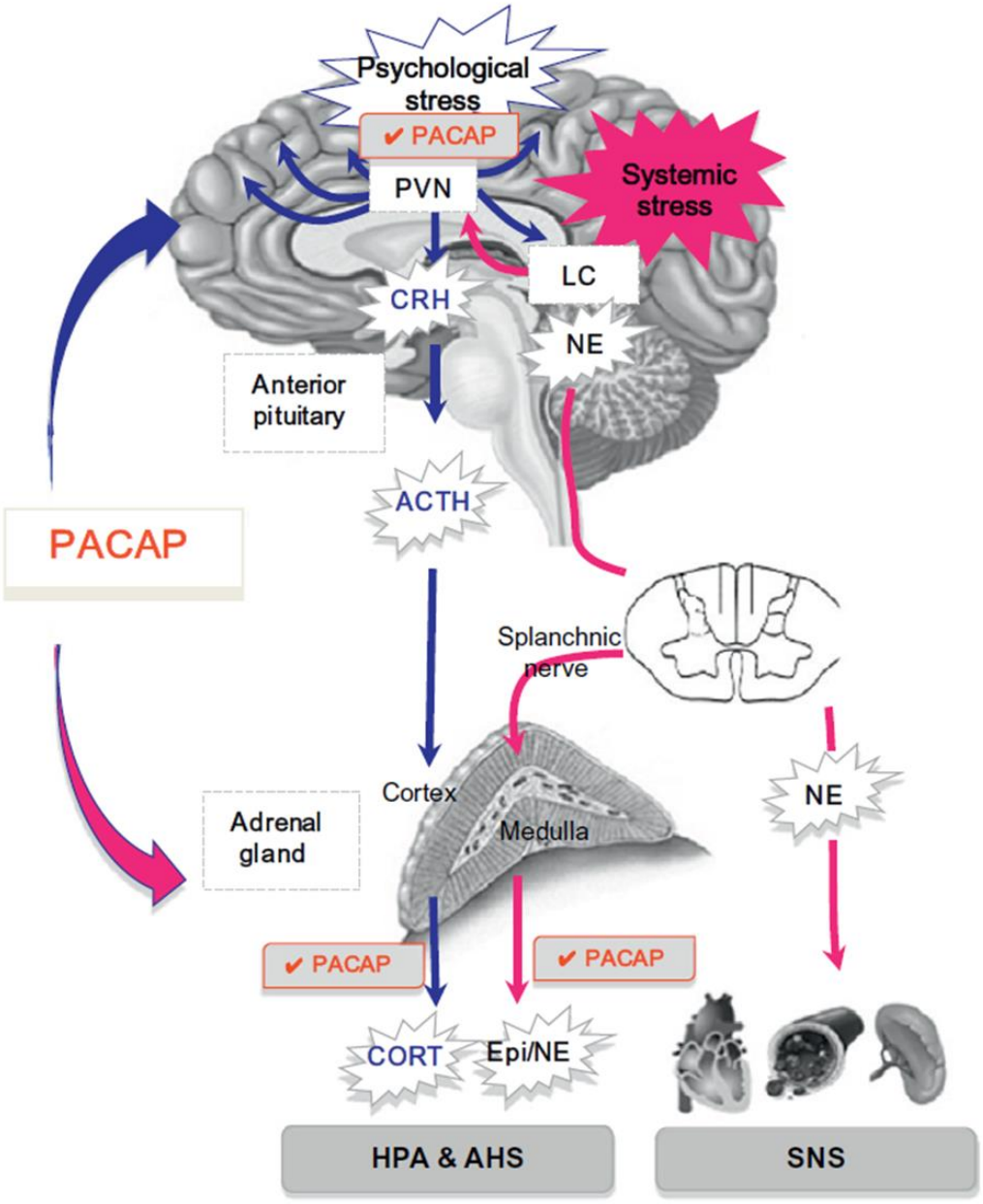


Figure 3: Hypothalamic-pituitary-adrenal (HPA) axis regulation of the stress response. The pituitary adenylate cyclase-activating polypeptide (PACAP) binds to PACAP receptor in the paraventricular nucleus (PVN) of the hypothalamus. The hypothalamus releases corticotropin-releasing hormone (CRH) which signals the pituitary gland to secrete adrenocorticotropic hormone (ACTH) and the adrenal cortex to release cortisol (CORT). Cortisol binds to glucocorticoid receptors in the hypothalamus and reduces CRH and ACTH secretion as part of the negative feedback loop of the HPA-axis. PACAP binding in the hypothalamus also signals the release of norepinephrine (NE) via the locus coeruleus (LC) to the effector organs of the Splanchnic nerve. The adrenal medulla, as part of the adrenal hormone system (AHS), releases NE and epinephrine in the bloodstream to sustain the sympathetic nervous system (SNS) response. Reprinted from "Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP): A Master Regulator in Central and Peripheral Stress Responses" by T Mustafa, 2013, *Advances in Pharmacology, 68*, p. 447, Copyright 2013 by Elsevier Incorporated [344].

1.4.2.5. The central nervous, endocrine and immune systems

Several studies have reported a link between PTSD and comorbid metabolic, cardiovascular, respiratory, gastrointestinal, inflammatory and autoimmune disease [140,345–352]. The relationship between PTSD and comorbid disease may in part be explained by alterations to the immune system [96]. A recent review of proinflammatory responses in PTSD found that interleukin 1 beta (IL-1B), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-a) and C-reactive protein (CRP) blood levels are generally increased in participants with PTSD compared to controls [353]. One prospective study found that CRP increased in relation to PTSD symptoms in male veterans [354]. Cross-sectional studies have found that there are no differences in proinflammatory markers between participants who recovered from PTSD and healthy controls, suggesting that proinflammatory markers covary with PTSD symptoms [355,356]. Other studies have shown that pre-existing alterations to the immune response and increased inflammation (due to genetic disposition, pre-existing disease, lifestyle factors, childhood trauma etc.) may be a risk factor for the development of PTSD following trauma exposure in adulthood [140,357–359].

The inflammatory response reported in PTSD studies is thought to be driven by the synthesis and secretion of proinflammatory cytokines [360]. Proinflammatory cytokines are secreted by various cells (e.g. macrophages, lymphocytes, endothelial cells, microglia, astrocytes and neurons) and is responsible for protection against pathogens by regulating systemic inflammation, fever and cell death [361,362]. However, upregulation of proinflammatory cytokines in the absence of infection may result in damage to healthy peripheral tissue and damage to neurons and ganglia in the central nervous system [363].

Cortisol is an immunosuppressant and downregulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), a transcription factor involved in the synthesis of cytokines [364]. The decreased levels of cortisol observed in PTSD studies may explain the increased synthesis of proinflammatory cytokines and the inflammatory state observed in PTSD studies [353,365]. A hyperactive SNS may further enhance cytokine synthesis and neuroinflammation [353,366] since NE released form the adrenal medulla upregulates NF-kB and the release of proinflammatory cytokines in the peripheral system [140,357]. Cytokine syntheses from microglia and astrocytes in brain regions (including the hypothalamus) with NE neuronal projections may result in neuroinflammation, neuronal damage and neuronal death (see Figure 4) [353,367].

One methylation study found that veterans with PTSD showed significantly decreased methylation levels (chr5:159314783-159330473, promoter region) of the gene coding the

proinflammatory cytokine interleukin 12 *(IL12)*, compared to a group of healthy controls not exposed to combat trauma [184]. Another study found that veterans with PTSD showed a significant increase in methylation of the proinflammatory cytokine interleukin 18 (*IL18)* from pre- to post-deployment [368]. Decreased methylation of the interferon-inducible protein / absent in melanoma 2 *(AIM2)* gene (chr1:159046973, TSS1500, promoter associated) has been associated with increased *AIM2* expression [369], increased CRP [175,369] and increased PTSD symptom severity in a veteran sample (Miller et al., 2018). AIM2 is involved in tumour reversion, control of cell proliferation and activation of the immune response and is mediated by the release of proinflammatory cytokines [175,369].

Differential methylation of genes involved in apoptosis and programmed cell death e.g. toll like receptor 8 (*TLR8*; chrX:12924783, not otherwise specified) [168]; mannosidase alpha class 2c member 1 (*MAN2C1*; chr15:75661449, island in promoter region) [185] and C-type lectin domain containing 9A (*CLEC9A*; chr12:10183364, not otherwise specified) [168] have also been associated with PTSD. TLR8 recognises the microbial structure of pathogens and promotes apoptosis of infected cells [370,371]. Decreased expression of *MAN2C1* by compromised cells signal the binding of cytotoxic T lymphocytes which initiates programmed cell death [372]. CLEC9A detects compromised cells marked for early cell death and regulates adaptive immunity to the pathogen that triggered cell death [373,374].

Dedicator of cytokinesis 2 (DOCK2) is a neurological immune regulator of phagocytes and its function is mediated by cytokines released from microglia [375]. One study found that decreased methylation (chr5:169068404, 5'UTR, promoter associated) of *DOCK2* was associated PTSD in a veteran sample [164]. Decreased methylation was also associated with increased expression of the gene in the same sample [164]. Increased *DOCK2* expression may result in neuroinflammation and toxicity (as is the case in Alzheimer's disease) [376] and may explain the previously identified link between PTSD and increased lifetime risk of early cognitive decline and dementia [164,377,378].

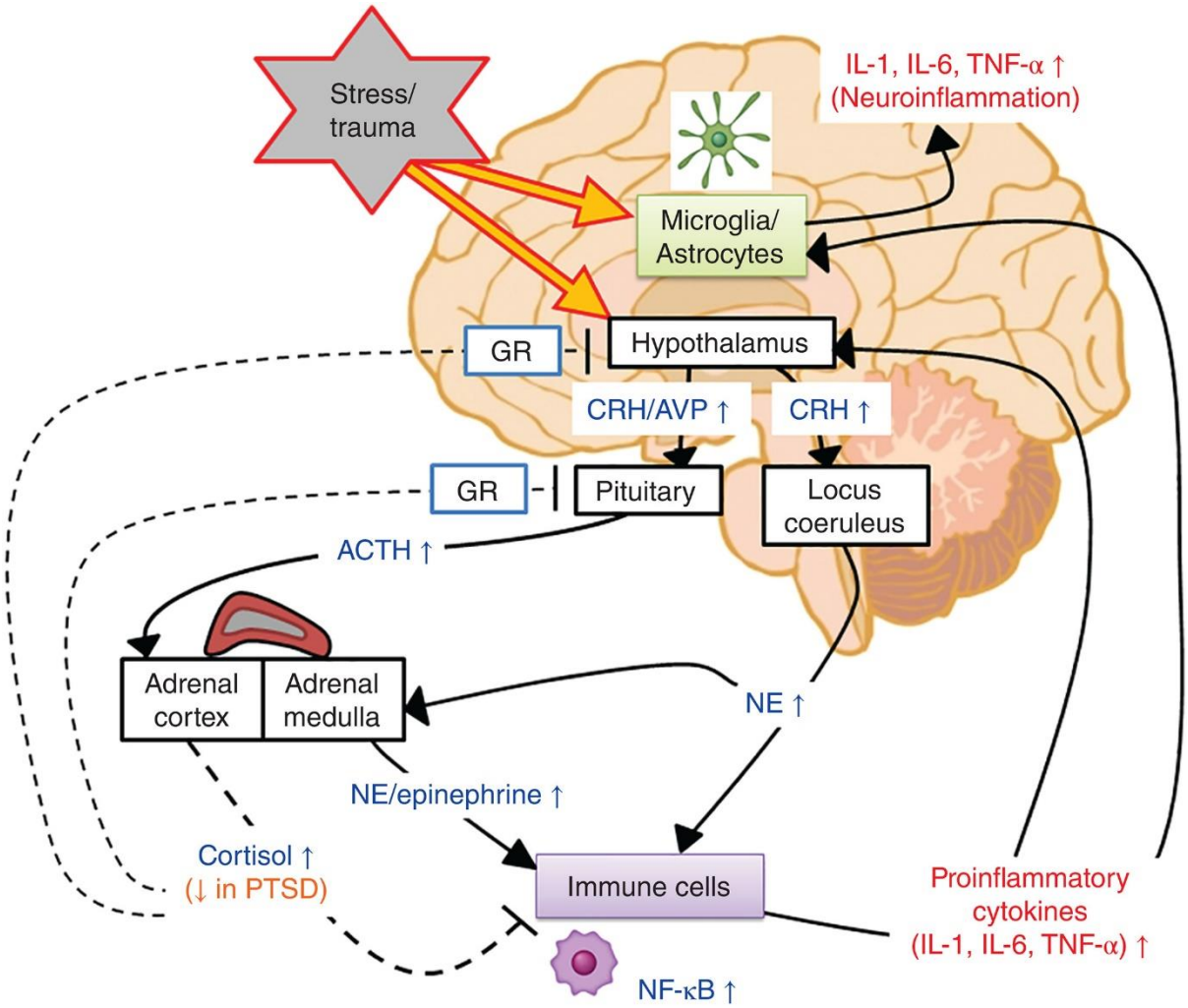


Figure 4: Interaction between the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system and the immune system. The hypothalamus releases corticotropin-releasing hormone (CRH) which signals the pituitary gland to secrete adrenocorticotropic hormone (ACTH) and the adrenal cortex to release cortisol. A dysregulated HPA-axis results in reduced cortisol secretion in posttraumatic stress disorder (PTSD). Reduced binding of cortisol to glucocorticoid receptors (GRs) in the hypothalamus and pituitary gland disrupts the negative feedback loop of the HPA-axis. Reduced cortisol (an immunosuppressant) increases nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activity and synthesis of proinflammatory cytokines e.g. interleukin 1, interleukin 6, tumour necrosis factor-α (TNFα). Increased release of norepinephrine (NE) from the adrenal medulla also upregulates NF-kB. NE neuronal projection (originating from the locus coeruleus) to brain regions associated with the HPA-axis increases cytokine synthesis from microglia and astrocytes resulting in neuroinflammation, neuronal damage and neuronal death. Reprinted from "Inflammation and post-traumatic stress disorder" by H Hori, Y Kim, 2019, *Psychiatric and Clinical Neurosciences, 73*, p. 146, Copyright 2019 by Wiley Online Library [353].

The neuroendocrinological characteristics of PTSD overlap to a large degree with those of metabolic syndrome (MetS) and cardiovascular disease [350,379]. PTSD, MetS and cardiovascular disease also share common metabolic characteristics that can be traced back to HPA-axis dysregulation (e.g. altered cortisol secretion, insulin resistance, glucose intolerance, hypertriglyceridemia, increased visceral adipose tissue, altered adipose hormone secretion) and altered SNS functioning (e.g. increased heart rate, hypertension, increased circulating NE) [350,353]. The overlap between PTSD, MetS and cardiovascular disease may be explained by underlying immune and inflammatory mechanisms such as chronic low-grade inflammation caused by increased synthesis of proinflammatory cytokines [380,381]. Differential methylation of genes related to central nervous system, endocrine system and immune system functioning likely modulate the phenotypic presentation of PTSD and associated diseases, although further research is needed to uncover the shared epigenetic alterations in PTSD, MetS and cardiovascular disease [138,164,167,170,174].

1.4.2.6. Confounding factors in methylation studies

Various environmental and biological factors, including age, shared ethnicity, gender, cell type composition, pharmaceuticals, toxins, metabolic disease, HIV infection and childhood trauma have been associated with differential methylation[382,383]. These confounding factors are discussed below and will be controlled for in all analyses related to methylation outcomes in the chapters to follow (details provided under section 1.5 – rationale and objectives).

1.4.2.6.1. Age

Chronological age has been linked to differentially methylated genes in various genome-wide studies which may explain the phenotypic presentation of aging and age associated increased risk for diseases [384–389]. Aging has been associated with decreased global methylation as well as differential methylation of genes associated with cell proliferation, tumour promoters and tumour suppressors [390]. Controlling for the effect of age in relation to methylation changes is therefore important in PTSD studies to distinguish between methylation changes associated with age and those associated with PTSD [390].

1.4.2.6.2. Shared ethnicity

Shared ethnicity has been linked to differential global methylation levels and distinct epigenome-wide profiles [391,392]. This may be due to inherited genetic and epigenetic alteration or due to shared environmental exposures such as air pollution, tobacco smoke, poverty etc.

[392,393]. Methylation profiles may also explain the ethnicity-related increased risk for specific diseases such as cardiovascular disease, metabolic disease, cancer and neurodegenerative diseases [392,394].

1.4.2.6.3. Gender

Significant differences in brain morphology, neurochemical activity and cognitive functions have been identified between men and women and may explain the increased risk for PTSD in women [395,396]. These differences may be due to epigenetic differences governing sexual differentiation (e.g. testosterone, oestrogen, steroid receptors, binding proteins) and their impact on neurological and endocrine functioning [395,397]. Similarly, female-specific methylation changes related to pregnancy and lactation have been reported and should be considered when investigating methylation profiles in relation to disease [398].

1.4.2.6.4. Cell type composition

Methylation also mediates cell differentiation and functional specialisation by activating tissue-specific genes and their expression [399]. The majority of human epigenetic studies investigating PTSD aetiology have relied on blood samples to identify differentially methylated genes, given that the procedure is minimally invasive and neuronal tissue is inaccessible in living participants [138]. However, methylation levels in blood may not be representative of methylation levels in brain tissue [400,401]. Some studies have reported a high correlation between overall average methylation at CpG sites in blood vs brain tissue, but the correlation becomes increasingly smaller when investigating specific genomic regions or individual CpG sites [400,402]. One review study found that the position of a CpG site in the gene is relatively important when investigating methylation levels given that blood methylation levels in CpG islands were generally highly correlated with methylation levels in brain tissue [401]. However, the long-term aim of epigenetic studies is generally to collate findings and identify potential blood biomarkers (since brain tissue cannot be used to assess risk) of psychiatric disorders and using this information to prioritise and individualise treatment according to individual risk profiles [403]. There are also studies that have assessed the correlation between blood and brain methylation and created online tools that can be used to support the relevance of CpG site-specific differential methylation findings in blood in relation to differentially methylation brain tissue [400,402,404,405].

Compared to other sample types (e.g. saliva and buccal mucosa), blood samples reflect brain methylation levels more accurately [402]. However, whole blood in itself is not homogenous

in cell type composition. It contains varying numbers of neutrophils, lymphocytes, monocytes, eosinophils and basophils each with different methylation profiles [406,407]. Using laboratory techniques such as flow cytometry can be implemented to identify and count specific blood cell types in induvial samples, and this can be controlled for in the methylation analysis [408]. As an alternative, cell type composition can be controlled for using reference-based methods i.e. based on prior findings and characteristics of the study participants [409]. The Houseman algorithm is a popular referenced-based method used in methylation studies to control for the heterogeneity of methylation levels across cell types [410]. Cell type composition can compromise the findings in methylation studies and controlling for variation in cell type is therefore important [409].

1.4.2.6.5. Pharmaceuticals

Various common pharmaceuticals have been found to exert their effects through alterations in DNA methylation, chromatin structure, transcription factor activity and receptor expression [411]. Pharmaceuticals with known epigenetic interactions include those related to cardiovascular disease (hydralazine, procainamide, beta-blockers and statins); seizures and psychiatric disorders (valproic acid, neuroleptics, SSRIs, methylphenidate); skin disorders and cancer (methotrexate, thalidomide, isotretinoin, chemotherapeutics); antimalarials (chloroquine); general anaesthetics and hormone contraceptives [411]. Pharmaceutical confounding should be considered when investigating differential methylation in relation to disease. The inclusion of drug-naïve participants can overcome the confounding effects of pharmaceuticals.

1.4.2.6.6. Toxins

Cigarette smoke has been linked to reduced global methylation as well as genome-wide differentially methylated genes related to the metabolism of toxins [412]. Similarly, exposure to persistent organic pollutants such as insecticides, pesticides and by-products of industrial production processes have been linked to reduced global methylation and disruption of neurological, endocrine and intracellular pathways [413]. Alcohol dependence has also been associated with decreased global methylation levels and genome-wide differential methylation of genes associated with the dopaminergic system, neurotransmitters and inflammation [414,415]. The confounding effect of exposure to toxins should therefore be considered when investigating methylation in relation to PTSD.

1.4.2.6.7. Metabolic disease

Several previous studies have linked PTSD to an increased risk for metabolic disease including obesity, type 2 diabetes, hypertension, increased triglycerides and decreased HDL cholesterol [350,416]. The link between PTSD and metabolic disease is likely due to differential methylation of genes involved in mitochondrial function, metabolic and endocrine processes shared by both conditions [417]. Body mass index (BMI) as a measure of obesity has also been linked to genome-wide differentially methylated genes predominately linked to metabolic functions [418].

1.4.2.6.8. HIV infection

HIV infection is associated with increased risk for cardiovascular disease, cancers, diabetes and increased levels of triglycerides [419,420]. The relationship between HIV and increased risk for comorbid diseases is possibly mediated by epigenetic mechanisms [421]. Differential methylation of genes associated with transcription factors, chromatin remodelling, viral binding and viral transport have been associated with HIV infection as well as genes associated with the immune, metabolic and endocrine system [420,422–424]. Increased methylation age (5-14 years) have also been reported in HIV infected samples [389,420,425,426]. Differential methylation may also be a mechanism implemented by the virus in order to increase its survival and replication rate [420]. Controlling for the effect of HIV infection on methylation levels is therefore important when investigating diseases and disorders in populations with high HIV prevalence rates.

1.4.2.6.9. Childhood trauma

Exposure to childhood trauma is a risk factor for PTSD and various other psychiatric disorders [427,428]. The increased risk for psychiatric disorders is possibly mediated by epigenetic changes during critical periods of development as a result of childhood exposed to trauma [429]. Studies investigating healthy adults have reported significant differences in genome-wide DNA methylation profiles between those with and without childhood trauma [430] as well as differences in candidate genes associated with the nervous system and the endocrine system [431–437].

One study investigating genome-wide differential expression of genes in participants with PTSD and childhood trauma compared to those with PTSD without childhood trauma found that the gene expression profiles of these groups were almost completely unique with only 2% overlap [169]. Differential expression of *NR3C1* has also been reported between those with PTSD and childhood trauma compared to those with PTSD without childhood trauma [188]. Substantial evidence therefore links childhood trauma to differential methylation and controlling for the effect of childhood trauma when investigating methylation in relation to

psychiatric disorders is important given the effect of childhood trauma on the epigenome [427,428]. See Chapter 2 for a review of childhood trauma and differential methylation in healthy adults and adults with depression, PTSD, borderline personality disorder, bipolar disorder, generalised anxiety disorder, alcohol dependence, schizophrenia and suicide completers [438].

1.5. RATIONALE, AIM AND OBJECTIVES

The overarching aim of this study is to investigate the pre-, peri- and post-trauma risk and protective factors related to socio-demographic, psychological and epigenetic mechanisms in the aetiology and trajectory of PTSD following rape-exposure. The parent study, the Rape Impact Cohort Evaluation (RICE), from which the study participants and samples were drawn, included 852 rape-exposed women between 16 and 40 years of age [439]. Baseline assessments (including self-report questionnaires, physical examination and specimen collection) were completed within 20 days following the rape and were repeated at 3-months, 6-months, 12-months, 18-months, 24-months and 36-months post-rape. The sub-aims of the study which are elaborated as objectives below include (1) differential methylation associated with childhood trauma and adult mental health outcomes; (2) the psychosocial risk and protective factors associated with PTSD symptom trajectory; (3.1) epigenome-wide differentially methylated genes associated with PTSD; (3.2) differentially methylated candidate genes associated with PTSD; and (4) *FKBP5* intron 7 methylation and the trajectory of PTSD symptoms. Each objective is addressed in a separate chapter in the dissertation and presented in manuscript format. The rationale and approach used are included under each objective below.

Objective 1: Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review

Childhood trauma is an established risk factor for the development of a range of adult psychiatric disorders, but less is known about the epigenetic mechanisms potentially mediating the relationship between childhood trauma and adult mental health outcomes [27,440–445]. Identifying the differentially methylated genes that originated from exposure to childhood trauma and increased the risk for adult psychiatric disorders may provide insight into the underlying biological pathways affected and may aid in identifying epigenetic differences or similarities across disorders [139].

A systematic review of studies investigating differentially methylated genes in relation to childhood trauma was conducted to address this objective. Studies investigating healthy

adults and adults diagnosed with depression, PTSD, borderline personality disorder, bipolar disorder, generalised anxiety disorder, alcohol dependence, schizophrenia and suicide completers were included in the final pool of articles meeting inclusion/exclusion criteria. The article was published online in The World Journal of Biological Psychiatry in April 2019 and is presented in Chapter 2 of the dissertation [438].

Objective 2: Risk and protective factors affecting the symptom trajectory of posttraumatic stress disorder post-rape

The majority of studies investigating psychosocial risk and protective factors for PTSD have followed a cross-sectional retrospective design which limits causal inference and may be tainted by recall bias [446]. Few studies to date have investigated mental health outcomes in rape-exposed women using a longitudinal prospective design and those that have tend to pool attempted rape, completed rape and sexual assault together. Baseline pre-assault (age, education, employment, relationship status, HIV status, childhood trauma, cumulative lifetime trauma), assault (rape context e.g. number of perpetrators, relationship to perpetrator, coercion, use of a weapon, physical force, threatened murder, perceived death, repeat perpetrator, number of sexual acts, rape reported to police), and post-assault (perceived stress, rape stigma, alcohol use, depression, resilience, social support) psychosocial risk and protective factors were investigated in relation to change in PTSD symptom severity over time. Baseline, 3-month and 6-month post-rape self-report measures were used to address this objective. Participants meeting the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) [7] criteria for PTSD at baseline related to a trauma other than the rape were excluded (n = 215). Six hundred thirty-nine participants were retained and included in the analysis. The results are presented in Chapter 3 of the dissertation.

Objective 3.1: Genome-wide differentially methylated genes associated with posttraumatic stress disorder and longitudinal change in methylation in rape survivors

Twelve epigenome-wide association studies (EWASs) investigating PTSD as outcome have been published to date [160,161,170–173,162–169]. The majority of EWASs have investigated North American war veteran samples [160–162,165]. Those investigating civilian samples have included participants with a mixture of trauma types and most have not stratified their analysis by gender[167–170,173]. No EWAS to date has investigated PTSD symptoms in a rape-exposed female only cohort. The procedure followed to address Objective 3.1 was divided into two phases. Firstly, 48 participants (24 with PTSD and 24 without PTSD) at the 3-month post-rape

timepoint were matched on HIV status, age, education, income, childhood trauma, cumulative lifetime trauma, BMI and smoking status. All participants were female and of African ethnicity. Participants meeting baseline criteria for PTSD i.e. PTSD due to a trauma other than the rape were excluded as well as participants who were currently pregnant, lactating or participants who HIV seroconverted at any timepoint. The 48 samples were analysed using the HumanMethylation EPIC BeadChip [447] at the University of South California (USC) Epigenome Centre. The sample was limited to 48 participants given the prohibitively high cost of EWAS laboratory procedures.

Secondly, selected significant findings from the EWAS were investigated in the same sample of participants included in the EWAS study, using EpiTYPER analysis, in order to validate the results. To replicate the results, we included an additional 49 participants, independent of the participants included in the validation set, and investigated the selected significant EWAS findings, using the same method applied in the validation. We then combined the samples used in the validation and replication analyses and included methylation data from the baseline and 6-months post-rape timepoints to investigate methylation changes in relation to change in PTSD scores over time, in the combined set. The laboratory procedures were completed at Inqaba Biotec in South Africa using EpiTYPER Sequenom MassARRAY technology. The results of the EWAS, validation, replication and longitudinal findings are presented in Chapter 4 of the dissertation.

Objective 3.2: Replication of findings from prior epigenome-wide association studies and candidate gene studies investigating differentially methylated genes associated with PTSD

Various CpG sites within genes and in intergenic regions have been associated with PTSD in EWAS and candidate gene studies, but few studies have been able to replicate prior findings with the exception of some HPA-axis related genes e.g. *NR3C1* and *FKBP5* [138,279,448]. The variation in findings is likely due to the vast differences in sample size, gender ratio, age, ethnicity, cellular heterogeneity, study design, PTSD measures, adjusting for confounding variables and molecular technology used between studies [138,149]. Reporting significant and non-significant findings resulting from an EWAS and relating to genes investigated in prior studies may provide insight into the substantial, nominal, or negligible contribution of these genes in predicting risk for PTSD in a specific population and may guide future research. The results are presented in Chapter 4.

Objective 4: *FKBP5* intron 7 methylation and the trajectory of PTSD symptoms in rape-exposed women

Dysfunction of the HPA-axis has been implicated in the aetiology and trajectory of PTSD following trauma since it is the core system involved in the regulation of the stress response [138,139,448,449]. One of the most commonly investigated HPA-axis genes is the *FKBP5* gene [138]. FKBP5 is a co-chaperone and important functional regulator of the glucocorticoid receptor [330,332]. Intron 7 of the gene contains several know GREs and prior studies have reported differential methylation at some of these GREs in relation to PTSD [180,181,336,450]. Differential methylation of GREs in *FKBP5* may result in altered *FKBP5* expression and may have adverse effects on HPA-axis functioning and increase the risk for PTSD [336,451] [138]. We investigated a 467 bp region in intron 7 of the *FKBP5* gene which overlaps with the region investigated in prior studies [180,181,336,450]. The region includes eight CpG site, two of which are located in two separate GREs [336]. Only one of the CpG sites in this region is included on the HumanMethylation EPIC BeadChip (cg22363520) and this site is not located in a GRE [447]. *FKBP5* intron 7 methylation was investigated in the same sample of 96 participants described in objective 3. Longitudinal *FKBP5* methylation levels were investigated using the baseline, 3-month and 6-month post-rape timepoints. Longitudinal methylation levels were determined using EpiTYPER Sequenom MassARRAY technology.

A SNP in intron 2 of the *FKBP5* gene (rs1360780; 400pb from a GRE) has also been associated with altered HPA-axis functioning especially when considering the interaction between the T allele of the SNP and childhood trauma [336,449,452–456]. The T allele of rs1360780 increases *FKBP5* transcription, both in the absence and presence of glucocorticoid binding to GREs [336]. Increased expression likely occur as a result of the T allele and its adjacent alleles forming a TATA box, which acts as a transcription start site [337]. Binding of transcription factors in intron 2 results in the formation of a three-dimensional chromatin loop which brings the GREs of intron 2 and intron 7 into close contact and further enhances transcription of *FKBP5* [336,337]. We therefore included rs1360780 genotype and childhood trauma, in addition to *FKBP5* intron 7 methylation levels, as predictors of longitudinal change in PTSD symptoms scores. Genotyping was completed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. No studies to date have investigated childhood trauma, rs1360980 genotype, *FKBP5* intron 7 methylation and PTSD in rape-exposed women of African ethnicity exclusively, and only one study has investigated the relationship between *FKBP5* intron 7 methylation and PTSD longitudinally [450]. The results of this objective are presented in Chapter 5 of the dissertation.

1.6. CONCLUSION

This chapter provided a comprehensive review of the pre-trauma, peri-trauma and post-trauma risk and protective factors associated with the etiology and trajectory of PTSD and contextualized the aim and objectives of this study in the current literature. The chapters that follow cover the background, methodology, results and a discussion related to each of the aforementioned objectives. In the final chapter (Chapter 6), the findings of the study objectives are integrated to address the overarching aim of the study. Study limitations, clinical implications of the findings and recommendations for future research are also discussed.

REFERENCES

1. Atwoli L, Stein DJ, Williams DR, Mclaughlin KA, Petukhova M, Kessler RC. Trauma and posttraumatic stress disorder in South Africa : analysis from the South African Stress and Health Study. *BMC Psychiatry*. 2013;13(182).
2. Herman AA, Stein DJ, Seedat S, Heeringa SG, Moomal H, Williams DR. The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders. *South African Med J*. 2009;99(5):339-344. doi:10.7196/SAMJ.3374
3. Dückers MLA, Alisic E, Brewin CR. A vulnerability paradox in the cross-national prevalence of post-traumatic stress disorder. *Br J Psychiatry*. 2016;209(4):300-305. doi:10.1192/bjp.bp.115.176628
4. Atwoli L, Stein D, Koenen K, McLaughlin K. Epidemiology of posttraumatic stress disorder: prevalence, correlares and consequences. *Curr Opin Psychiatry*. 2015;28(4):307-311. doi:10.1097/YCO.0000000000000167.Epidemiology
5. Gradus JL. Prevalence and prognosis of stress disorders: A review of the epidemiologic literature. *Clin Epidemiol*. 2017;9:251-260. doi:10.2147/clep.s106250
6. Santiago PN, Ursano RJ, Gray CL, et al. A Systematic Review of PTSD Prevalence and Trajectories in DSM-5 Defined Trauma Exposed Populations : Intentional and Non-Intentional Traumatic Events. *PLoS One*. 2013;8(4):e59236. doi:10.1371/journal.pone.0059236
7. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, USA; 2013.
8. Shipherd JC, Stafford J, Tanner LR. Predicting alcohol and drug abuse in Persian Gulf War veterans: What role do PTSD symptoms play? *Addict Behav*. 2005;30(3):595-599. doi:10.1016/j.addbeh.2004.07.004
9. Sonne SC, Back SE, Zuniga CD, Randall CL, Brady KT. Gender Differences in Individuals with Comorbid Alcohol Dependence and Post-traumatic Stress Disorder. *Am J Addict*. 2003;12(5):412-423. doi:10.1111/j.1521-0391.2003.tb00484.x
10. Elklit A, Christiansen DM. ASD and PTSD in rape victims. *J Interpers Violence*. 2010;25(8):1470-1488. doi:10.1177/0886260509354587
11. Koss MP, Bailey JA, Yuan NP, Herrera VM, Lichter EL. Depression and PTSD in survivors of male violence: Research and training initiatives to facilitate recovery. *Psychol Women Q*. 2003;27(2):130-142. doi:10.1111/1471-6402.00093

12. Zinzow HM, Resnick HS, McCauley JL, Amstadter AB, Ruggiero KJ, Kilpatrick DG. Prevalence and risk of psychiatric disorders as a function of variant rape histories: Results from a national survey of women. *Soc Psychiatry Psychiatr Epidemiol*. 2012;47(6):893-902. doi:10.1007/s00127-011-0397-1
13. Kaminer D, Grimsrud A, Myer L, Stein D, Williams D. Risk for posttraumatic stress disorder associated with diffetent forms of interpersonal violence in South Africa. *Soc Sci Med*. 2008;67(10):1589-1595. doi:10.1016/j.socscimed.2008.07.023.RISK
14. Rodriguez P, Holowka DW, Marx BP. Assessment of posttraumatic stress disorder-related functional impairment: A review. *J Rehabil Res Dev*. 2012;49(5):649. doi:10.1682/JRRD.2011.09.0162
15. Hayes JP, VanElzakker MB, Shin LM. Emotion and Cognition Interactions in PTSD: A Review of Neurocognitive and Neuroimaging Studies. *Front Integr Neurosci*. 2012;6:1-14. doi:10.3389/fnint.2012.00089
16. South African Government Gazette. *Criminal Law (Sexual Offences and Related Matters) Amendment Act 32 of 2007*.; 2007. http://www.justice.gov.za/legislation/acts/2007-032.pdf.
17. Statistics South Africa. *Governance, Public Safety and Justice Survey: GPSJS 2018/2019*. Pretoria, South Africa; 2019.
18. Jewkes R, Abrahams N. The epidemiology of rape and sexual coercion in South Africa: An overview. *Soc Sci Med*. 2002;55(7):1231-1244. doi:10.1016/S0277-9536(01)00242-8
19. Machisa M, Jewkes R, Morna C, Rama K. *The War at Home*. Johannesburg, South Africa; 2011.
20. Vetten L. Rape and other forms of sexual violence in South Africa (Policy Brief 72). *Inst Secur Stud*. 2014;72(November):1-6. https://issafrica.org/research/policy-brief/rape-and-other-forms-of-sexual-violence-in-south-africa/download-policy-brief-72-pdf.
21. Kapp C. Rape on trial in South Africa. *Lancet*. 2006;367(9512):718-719. doi:10.1016/S0140-6736(06)68285-8
22. Jewkes R, Christofides N, Vetten L, Jina R, Sigsworth R, Loots L. Medico-legal findings, legal case progression, and outcomes in South African rape cases: Retrospective review. *PLoS Med*. 2009;6(10). doi:10.1371/journal.pmed.1000164
23. Sepeng NV, Makhado L. Present practices of rape care management in Thuthuzela Care Centres of the North West Province. *J Psychol Africa*. 2019;29(5):516-519.

doi:10.1080/14330237.2019.1677057

24. Mafani P, Lawrence B. *Report on the 1st Sexual Offences Indaba*.; 2009. https://www.npa.gov.za/sites/default/files/sexual-offences-ndaba-2008/SO Indaba Report.pdf.
25. Bougard NB, Booyens K. Adult female rape victims' views about the Thuthuzela Care Centre service delivery model. *Acta Criminol South African J Criminol*. 2015;(5):19-33.
26. Abrahams N, Jewkes R. Barriers to post exposure prophylaxis (PEP) completion after rape: A South African qualitative study. *Cult Heal Sex*. 2010;12(5):471-484. doi:10.1080/13691050903556316
27. Chen LP, Murad MH, Paras ML, et al. Sexual abuse and lifetime diagnosis of psychiatric disorders: Systematic review and meta-analysis. *Mayo Clin Proc*. 2010;85(7):618-629. doi:10.4065/mcp.2009.0583
28. Crocq MA, Crocq L. From shell shock and war neurosis to posttraumatic stress disorder: a history of psychotraumatology. *Dialogues Clin Neurosci*. 2000;2(1):47-55. http://www.ncbi.nlm.nih.gov/pubmed/22033462%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3181586.
29. Turnbull GJ. A review of post-traumatic stress disorder. Part I: Historical development and classification. *Injury*. 1998;29(2):87-91. doi:10.1016/S0020-1383(97)00131-9
30. Tatu L. Edgar Adrian (1889–1977) and Shell Shock Electrotherapy: A Forgotten History? *Eur Neurol*. 2018;79:106-107. doi:10.1159/000486762
31. Reid F. "His nerves gave way": Shell shock, history and the memory of the First World War in Britain. *Endeavour*. 2014;38(2):91-100. doi:10.1016/j.endeavour.2014.05.002
32. Skinner R, Kaplick PM. Cultural shift in mental illness: a comparison of stress responses in World War I and the Vietnam War. *JRSM Open*. 2017;8(12):205427041774606. doi:10.1177/2054270417746061
33. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 3rd ed. Arlington, USA; 1980.
34. Engel GL. The clinical application of the biopsychosocial model. *Am J Psychiatry*. 1980;137(5):535-544. http://jmp.oxfordjournals.org/.
35. Borrell-Carrio F, Suchman A, Epstein R. The Biopsychosocial Model 25 Years Later: Principles, Practice, and Scientific Inquiry. *Ann Fam Med*. 2004;2(6):576-582. doi:10.1370/afm.245.Department
36. Christopher M. A broader view of trauma: A biopsychosocial-evolutionary view of the

role of the traumatic stress response in the emergence of pathology and/or growth. *Clin Psychol Rev*. 2004;24(1):75-98. doi:10.1016/j.cpr.2003.12.003

37. Wafa MH, Viprey M, Magaud L, et al. Identification of biopSychoSocial factors predictive of post-traUmatic stress disorder in patients admitted to the Emergency department after a trauma (ISSUE): Protocol for a multicenter prospective study. *BMC Psychiatry*. 2019;19(163):1-11. doi:10.1186/s12888-019-2154-z
38. Engel G. The need for a new medical model: a challenge for biomedicine. *Science (80-)*. 1977;196(4286):129-136.
39. Baumeister A. The chlorpromazine enigma. *J Hist Neurosci*. 2013;22(1):14-29.
40. Duncan LE, Cooper BN, Shen H. Robust Findings From 25 Years of PTSD Genetics Research. *Curr Psychiatry Rep*. 2018;20(115):1-9.
41. Martin-Soelch C, Schnyder U. Editorial: Resilience and Vulnerability Factors in Response to Stress. *Front Psychiatry*. 2019;10(October):732. doi:10.3389/FPSYT.2019.00732
42. Ravindran LN, Stein MB. Pharmacotherapy of PTSD : Premises , principles , and priorities. *Brain Res*. 2009;1293:24-39. doi:10.1016/j.brainres.2009.03.037
43. Ben-Zeev D, Young M, Corrigan P. DSM-V and the stigma of mental illness. *J Ment Heal*. 2010;19(4):318-327.
44. Carlson EB, Palmieri PA, Field NP, Dalenberg CJ, Macia KS, Spain DA. Contributions of risk and protective factors to prediction of psychological symptoms after traumatic experiences. *Compr Psychiatry*. 2016;69:106-115. doi:10.1016/j.comppsych.2016.04.022
45. Lauth-Lebens M, Lauth G. Risk and Resilience Factors of Post-Traumatic Stress Disorder: A Review of Current Research. *Clin Exp Psychol*. 2016;02(02). doi:10.4172/2471-2701.1000120
46. Erickson LD, Hedges DW, Call VRA, Bair B. Prevalence of and factors associated with subclinical posttraumatic stress symptoms and ptsd in urban and rural areas of Montana: A cross-sectional study. *J Rural Heal*. 2013;29(4):403-412. doi:10.1111/jrh.12017
47. DiGangi J, Gomez D, Mendoza L, Jason L, Keys C, Koene K. Pretrauma risk factors for posttraumatic stress disorder: A systematic review of the literature. *Clin Psychol Rev*. 2013;33(16):728-744.
48. Voges MA, Romney DM. Risk and resiliency factors in posttraumatic stress disorder. *Ann Gen Hosp Psychiatry*. 2003;2:1-9. doi:10.1186/1475-2832-2-4

49. Holzinger A, Floris F, Schomerus G, Carta MG, Angermeyer MC. Gender differences in public beliefs and attitudes about mental disorder in western countries: A systematic review of population studies. *Epidemiol Psychiatr Sci*. 2012;21(1):73-85. doi:10.1017/S2045796011000552
50. Olff M. Sex and gender differences in post-traumatic stress disorder : an update. 2017;8. doi:10.3402/ejpt.v5.25547
51. Maeng LY, Milad MR. Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Horm Behav*. 2015;76:106-117. doi:10.1016/j.yhbeh.2015.04.002
52. Arata C. From child vicitm to adult victim: a model for predicting sexual revictimization. *Child Maltreat*. 2000;5(1):28-38.
53. Arata C. Child sexual abuse and sexual revictimization. *Clin Psychol Sci Pract*. 2002;9(2):135-164. doi:10.1093/clipsy/9.2.135
54. Classen CC, Palesh OG, Aggarwal R. Sexual revictimization: A review of the empirical literature. *Trauma, Violence, Abus*. 2005;6(2):103-129. doi:10.1177/1524838005275087
55. Desai S, Arias I, Thompson MP, Basile KC. Childhood victimization and subsequent adult revictimization assessed in a nationally representative sample of women and men. *Violence Vict*. 2002;17(6):639-653. doi:10.1891/vivi.17.6.639.33725
56. Ozer E, Best S, Lipsey T, Weis D. Predictors of Posttraumatic Stress Disorder and Symptoms in Adults : A Meta-Analysis Predictors of Posttraumatic Stress Disorder and Symptoms in Adults : *Psychol TraumaL Theory, Res Pract Policy*. 2008;(1):52-73. doi:10.1037/1942-9681.S.1.3
57. Fairbank JA, Fairbank DW. Epidemiology of child traumatic stress. *Curr Psychiatry Rep*. 2009;11(4):289-295. doi:10.1007/s11920-009-0042-9
58. Nishith P, Mechanic MB, Resick PA. Prior interpersonal trauma: The contribution to current PTSD symptoms in female rape victims. *J Abnorm Psychol*. 2000;109(1):20-25. doi:10.1037/0021-843X.109.1.20
59. Bryant-Davis T, Ullman SE, Tsong Y, Tillman S, Smith K. Struggling to survive: Sexual assault, poverty, and mental health outcomes of african american women. *Am J Orthopsychiatry*. 2010;80(1):61-70. doi:10.1111/j.1939-0025.2010.01007.x
60. Cloitre M, Stolbach B, Herman J, et al. A developmental approach to complex PTSD: childhood and adult cumulative trauma as predictors of symptom complexity. *J Trauma Stress*. 2009;22(5):293-300. doi:10.1002/jts

61. Ullman S, Filipas H, Townsend S, Starzynski L. Psychosocial correlates of PTSD symptom severity in sexual assault survivors. *J Trauma Stress*. 2007;20(5):821-831. doi:10.1002/jts.20290

62. Wasco SM. Conceptualizing the Harm done by Rape: Applications of Trauma Theory to Experiences of Sexual Assault. *Trauma, Violence, Abus*. 2003;4(4):309-322. doi:10.1177/1524838003256560

63. Weich S, Patterson J, Shaw R, Stewart-Brown S. Family relationships in childhood and common psychiatric disorders in later life: systematic review of prospective studies. *Br J Psychiatry*. 2009;194(5):392-398. doi:10.1192/bjp.bp.107.042515

64. Riggs SA. Childhood Emotional Abuse and the Attachment System Across the Life Cycle: What Theory and Research Tell Us. *J Aggress Maltreat Trauma*. 2010;19(1):5-51. doi:10.1080/10926770903475968

65. Pechtel P, Pizzagalli D. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology (Berl)*. 2011;214(1):55-70.

66. Weathers F, Blake D, Schnurr P, Kaloupek D, Marx B, Keane T. *The Life Events Checklist for DSM-5 (LEC-5)*. USA: National Center for PTSD; 2013.

67. Ogle CM, Rubin DC, Siegler IC. Cumulative exposure to traumatic events in older adults. *Aging Ment Heal*. 2014;18(3):316-325. doi:10.1080/13607863.2013.832730

68. Elklit A, Christiansen DM. Risk factors for posttraumatic stress disorder in female help-seeking victims of sexual assault. *Violence Vict*. 2013;28(3):552-568. doi:10.1891/0886-6708.09-135

69. Möller AT, Bäckström T, Söndergaard HP, Helström L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One*. 2014;9(10):1-9. doi:10.1371/journal.pone.0111136

70. Demorrow S. Role of the hypothalamic–pituitary–adrenal axis in health and disease. *Int J Mol Sci*. 2018;19(4). doi:10.3390/ijms19040986

71. Kuhlman KR, Chiang JJ, Horn S, Bower JE. Developmental psychoneuroendocrine and psychoneuroimmune pathways from childhood adversity to disease. *Neurosci Biobehav Rev*. 2017;80(April):166-184. doi:10.1016/j.neubiorev.2017.05.020

72. Sheerin C, Lind M, Bountress K, Nugent N, Amstadter A. The genetics and epigenetics of PTSD: overview, recent advances, and future directions. *Curr Opin Psychol*. 2017;5(5):395-404. doi:10.1021/acssynbio.5b00266.Quantitative

73. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic

diseases, and decrements in health: results from the World Health Survey. *Lancet*. 2007;370:29-32.

74. Brandt R. The mental health of people living with HIV / AIDS in Africa : a systematic review The mental health of people living with HIV / AIDS in Africa : a systematic review. *African J AIDS Res*. 2009;8(2):123-133. doi:10.2989/AJAR.2009.8.2.1.853
75. Welch J, Mason F. Rape and sexual assault. *Clin Rev*. 2007;334(June):1154-1158. doi:10.1136/bmj.39211.403970.BE
76. Statistics South Africa. *2019 Mid-Year Population Estimates*.; 2019.
77. Koenen KC, Fu QJ, Ertel K, et al. Common genetic liability to major depression and posttraumatic stress disorder in men. *J Affect Disord*. 2008;105:109-115. doi:10.1016/j.jad.2007.04.021
78. True W, Rice J, Eisen S, et al. A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch Gen Psychiatry*. 1993;50(4):257-264. doi:10.1001/archpsyc.1993.01820160019002
79. Wolf EJ, Mitchell KS, Koenen KC, Miller MW. Combat exposure severity as a moderator of genetic and environmental liability to post-traumatic stress disorder. *Psychol Med*. 2014;44:1499-1509. doi:10.1017/S0033291713002286
80. Wolf EJ, Miller MW, Krueger RF, Lyons MJ. Posttraumatic stress disorder and the genetic structure of comorbidity. *J Abnorm Psychol*. 2011;119(2):320-330. doi:10.1037/a0019035.Posttraumatic
81. Stein MB, Jang KL, Ph D, et al. Genetic and Environmental Influences on Trauma Exposure and Posttraumatic Stress Disorder Symptoms : A Twin Study. *Am J Psychiatry*. 2002;159:1675-1681.
82. Sartor C, McCutcheon V, Pommer N, et al. Common genetic and environmental contributions to posttraumatic stress disorder and alcohol dependence in young women. *Psychol Med*. 2011;41(7):1497-1505. doi:10.1017/S0033291710002072.Common
83. Scheeringa MS, Zeanah CH, Cohen JA. PTSD in children and adolescents: Toward an empirically based algorithm a. *Depress Anxiety*. 2011;28(9):770-782. doi:10.1002/da.20736
84. Twardosz S, Lutzker JR. Child maltreatment and the developing brain: A review of neuroscience perspectives. *Aggress Violent Behav*. 2010;15(1):59-68. doi:10.1016/j.avb.2009.08.003
85. Christiansen DM, Hansen M. Accounting for sex differences in PTSD: A multi-

variable mediation model. *Eur J Psychotraumatol*. 2015;6:23068.

86. Speed D, Cai N, Consortium U, Johnson MR, Nejentsev S, Balding DJ. Articles Reevaluation of SNP heritability in complex human traits. *Nat Publ Gr*. 2017;49(7). doi:10.1038/ng.3865
87. Duncan L, Ratanatharathorn A, Aiello AE, et al. Largest GWAS of PTSD (N = 20 070) yields genetic overlap with schizophrenia and sex differences in heritability. *Mol Psychiatry*. 2018;23:666-673. doi:10.1038/mp.2017.77
88. Nievergelt CM, Maihofer A, Torsten K, et al. International meta-analysis of PTSD genome-wide association studies identi fi es sex- and ancestry- speci fi c genetic risk loci. *Nat Commun*. 2019;10(4558). doi:10.1038/s41467-019-12576-w
89. Kose S, Mandiracioglu A, Mermut G, Kaptan F, Ozbel Y. The Social and Health Problems of People Living with HIV/AIDS in Izmir, Turkey. *Eurasian J Med*. 2012;44(1):32-39. doi:10.5152/eajm.2012.07
90. Schoemaker J, Twikirize J. A life of fear: Sex workers and the threat of HIV in Uganda. *Int J Soc Welf*. 2012;21(2):186-193. doi:10.1111/j.1468-2397.2010.00770.x
91. Applebaum A, Bedoya C, Hendriksem E, Wilkinson J, Safren S, O'Cleirigh C. Future directions for interventions targeting PTSD in HIV-infected adults. *J Assoc Nurses AIDS Care*. 2015;26(2):127-138. doi:10.1016/j.jana.2014.11.001.Future
92. Young C. Understanding HIV-related posttraumatic stress disorder in South Africa : a review and conceptual framework Understanding HIV-related posttraumatic stress disorder in South Africa : a review and conceptual framework. *African J AIDS Res*. 2011;10(2):139-148. doi:10.2989/16085906.2011.593376
93. Sherr L, Nagra N, Kulubya G, et al. HIV infection associated post-traumatic stress disorder and post-traumatic growth – A systematic review. *Psychol Health Med*. 2011;16(5). doi:10.1080/13548506.2011.579991
94. Machtinger EL, Wilson TC, Haberer JE, Weiss DS. Psychological trauma and PTSD in HIV-positive women: A meta-analysis. *AIDS Behav*. 2012;16(8):2091-2100. doi:10.1007/s10461-011-0127-4
95. Breet E, Kagee A, Seedat S. HIV-related stigma and symptoms of post- traumatic stress disorder and depression in HIV- infected individuals : does social support play a mediating or moderating role ? *AIDS Care*. 2014;26(8):947-951. doi:10.1080/09540121.2014.901486
96. Neigh GN, Rhodes ST, Valdez A, Jovanovic T. Neurobiology of Disease PTSD co-morbid with HIV : Separate but equal , or two parts of a whole? *Neurobiol Dis*.

2016;92:116-123. doi:10.1016/j.nbd.2015.11.012

97. Iversen AC, Mrcpsych M, Fear NT, et al. Europe PMC Funders Group Risk factors for Post Traumatic Stress Disorder amongst United Kingdom Armed Forces personnel. *Psychol Med*. 2013;38(4):511-522. doi:10.1017/S0033291708002778.Risk
98. Kelley L, Weathers F, McDevitt-Murphy M, Eakin D, Flood A. A comparison of PTSD symptom patterns in three types of civilian trauma. *J Trauma Stress*. 2016;29(August):293-300. doi:10.1002/jts
99. Shakespeare-Finch J, Armstrong D. Trauma type and posttrauma outcomes: Differences between survivors of motor vehicle accidents, sexual assault, and bereavement. *J Loss Trauma*. 2010;15(2):69-82. doi:10.1080/15325020903373151
100. Frans O, Rimmö PA, Åber L, Fredrikson M. Trauma exposure and post-traumatic stress disorder in the general population. *Acta Psychiatr Scand*. 2005;111(4):291-299. doi:10.1111/j.1600-0447.2004.00463.x
101. Brewin CR, Andrews B, Valentine JD. Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol*. 2000;68(5):748-766. doi:10.1037/0022-006X.68.5.748
102. Besser A, Neria Y, Haynes M. Adult attachment, perceived stress, and PTSD among civilians exposed to ongoing terrorist attacks in Southern Israel. *Pers Individ Dif*. 2009;47(8):851-857. doi:10.1016/j.paid.2009.07.003
103. Sorsdahl KR, Stein DJ. Knowledge of and stigma associated with mental disorders in a South African community sample. *J Nerv Ment Dis*. 2010;198(10):742-747. doi:10.1097/NMD.0b013e3181f4b2d7
104. Campbell DG, Felker BL, Liu CF, et al. Prevalence of depression-PTSD comorbidity: Implications for clinical practice guidelines and primary care-based interventions. *J Gen Intern Med*. 2007;22(6):711-718. doi:10.1007/s11606-006-0101-4
105. Nixon RD V, Resick PA, Nishith P. An exploration of comorbid depression among female victims of intimate partner violence with posttraumatic stress disorder. *J Affect Disord*. 2004;82(2):315-320. doi:10.1016/j.jad.2004.01.008
106. Stein MB, Kennedy C. Major depressive and post-traumatic stress disorder comorbidity in female victims of intimate partner violence. *J Affect Disord*. 2001;66(2-3):133-138. doi:10.1016/S0165-0327(00)00301-3
107. Schumm JA, Briggs-Phillips M, Hobfoll SE. Cumulative interpersonal traumas and social support as risk and resiliency factors in predicting PTSD and depression among inner-city women. *J Trauma Stress*. 2006;19(6):825-836. doi:10.1002/jts.20159

108. Guay S, Billette V, Marchand A. Exploring the links between posttraumatic stress disorder and social support: processes and potential research avenues. *J Trauma Stress*. 2016;19(3):327-338. doi:10.1002/jts

109. Levine S, Laufer A, Stein E, Hamama-Raz Y, Solomon Z. Examining the relationship between resilience and posttraumatic growth. *J Trauma Stress*. 2009;22(4):282-286. doi:10.1002/jts

110. Waysman M, Schwarzwald J, Solomon Z. Hardiness: An examination of its relationship with positive and negative long term changes following trauma. *J Trauma Stress*. 2001;14(3):531-548. doi:10.1023/A:1011112723704

111. Matud MP. Gender differences in stress and coping styles. *Pers Individ Dif*. 2004;37(7):1401-1415. doi:10.1016/j.paid.2004.01.010

112. Pierrehumbert B, Torrisi R, Glatz N, Dimitrova N, Heinrichs M, Halfon O. The influence of attachment on perceived stress and cortisol response to acute stress in women sexually abused in childhood or adolescence. *Psychoneuroendocrinology*. 2009;34(6):924-938. doi:10.1016/j.psyneuen.2009.01.006

113. Carretta CM, Burgess AW, DeMarco R. To Tell or Not to Tell. *Violence Against Women*. 2016;22(13):1499-1518. doi:10.1177/1077801215627507

114. Moor A, Farchi M. Is rape-related self blame distinct from other post traumatic attributions of blame? A comparison of severity and implications for treatment. *Women Ther*. 2011;34(4):447-460. doi:10.1080/02703149.2011.591671

115. Ullman SE, Filipas HH, Townsend SM, Starzynski LL. The role of victim-offender relationship in women's sexual assault experiences. *J Interpers Violence*. 2006;21(6):798-819. doi:10.1177/0886260506288590

116. Ullman S, Filipas H. Predictors of PTSD symptom severity and social reactions in sexual assault victims. *J Trauma Stress*. 2001;14(2):369-389. doi:10.1023/A

117. Flouri E. Post-Traumatic Stress Disorder (PTSD): What we have learned and what we still have not found out. *J Interpers Violence*. 2005;20(4):373-379. doi:10.1177/0886260504267549

118. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

119. Driessen M, Schulte S, Luedecke C, et al. Trauma and PTSD in patients with alcohol, drug, or dual dependence: A multi-center study. *Alcohol Clin Exp Res*. 2008;32(3):481-488. doi:10.1111/j.1530-0277.2007.00591.x

120. Kaysen D, Neighbors C, Martell J, Fossos N, Larimer ME. Incapacitated rape and alcohol use: A prospective analysis. *Addict Behav*. 2006;31(10):1820-1832. doi:10.1016/j.addbeh.2005.12.025

121. De Bellis MD. Developmental traumatology: A contributory mechanism for alcohol and substance use disorders. *Psychoneuroendocrinology*. 2002;27(1-2):155-170. doi:10.1016/S0306-4530(01)00042-7

122. Breslau N. Outcomes of posttraumatic stress disorder. *J Clin Psychiatry*. 2001;62(SUPPL. 17):55-59.

123. Waldrop AE, Back SE, Verduin ML, Brady KT. Triggers for cocaine and alcohol use in the presence and absence of posttraumatic stress disorder. *Addict Behav*. 2007;32(3):634-639. doi:10.1016/j.addbeh.2006.06.001

124. Acierno R, Resnick H, Kilpatrick DG, Saunders B, Best CL. Risk factors for rape, physical assault, and posttraumatic stress disorder in women: Examination of differential multivariate relationships. *J Anxiety Disord*. 1999;13(6):541-563. doi:10.1016/S0887-6185(99)00030-4

125. Plichta SB, Falik M. Prevalence of violence and its implications for women's health. *Women's Heal Issues*. 2001;11(3):244-258. doi:10.1016/S1049-3867(01)00085-8

126. Ullman SE, Brecklin LR. Sexual assault history and health-related outcomes in a national sample of women. *Psychol Women Q*. 2003;27(1):46-57. doi:10.1111/1471-6402.t01-2-00006

127. Littleton H. An evaluation of the coping patterns of rape victims: Integration with a schema-based information-processing model. *Violence Against Women*. 2007;13(8):789-801. doi:10.1177/1077801207304825

128. Andrews B, Brewin CR, Rose S. Gender, social support, and PTSD in victims of violent crime. *J Trauma Stress*. 2003;16(4):421-427. doi:10.1023/A:1024478305142

129. Hoge E, Austin E, Pollack M. Resilience: research evidence and conceptual considerations for posttraumatic stress disorder. *Depress Anxiety*. 2007;24:139-152. doi:10.1002/da

130. Connor KM, Davidson JRT. Development of a new Resilience scale: The Connor-Davidson Resilience scale (CD-RISC). *Depress Anxiety*. 2003;18(2):76-82. doi:10.1002/da.10113

131. Edwards D, Sakasa P, Van Wyk G. Trauma, resilience and vulnerability to PTSD: A review and clinical case analysis. *J Psychol Africa*. 2005;15(2):143-153. doi:10.4314/jpa.v15i2.30652

132. Bowland S, Biswas B, Kyriakakis S, Edmond T. Transcending the negative: Spiritual struggles and resilience in older female trauma survivors. *J Relig Spiritual Aging*. 2011;23(4):318-337. doi:10.1080/15528030.2011.592121
133. Agaibi CE, Wilson JP. Trauma, PTSD, and Resilience: A Review of the Literature. *Trauma, Violence, Abus*. 2005;6(3):195-216. doi:10.1177/1524838005277438
134. Walsh K, Blaustein M, Knight W, Spinazzola J, van der Kolk B. Resiliency factors in the relation between childhood sexual abuse and adulthood sexual assault in college-age women. *J Child Sex Abus*. 2007;16(1):1-17. doi:10.1300/J070v16n01
135. Hauck S, Schestatsky S, Terra L, Kruel L, Helena Freitas Ceitlin L. Parental bonding and emotional response to trauma: A study of rape victims. *Psychother Res*. 2007;17(1):83-90. doi:10.1080/10503300500477596
136. Borja S, Callahan J, Long P. Positive and negative adjustment and social support of sexual assault survivors. *J Trauma Stress*. 2006;19(6):905-914. doi:10.1002/jts
137. Suzuki SL, Geffner R, Bucky SF. The experiences of adults exposed to intimate partner violence as children: An exploratory qualitative study of resilience and protective factors. *J Emot Abus*. 2008;8(1-2):103-121. doi:10.1080/10926790801984523
138. Morrison FG, Miller MW, Logue MW, Assef M, Wolf EJ. DNA methylation correlates of PTSD: Recent findings and technical challenges. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;90(November 2018):223-234. doi:10.1016/j.pnpbp.2018.11.011
139. Vinkers CH, Kalafateli AL, Rutten BP, et al. Traumatic stress and human DNA methylation: A critical review. *Epigenomics*. 2015;7(4):593-608. doi:10.2217/epi.15.11
140. Neigh GN, Ali FF. Co-morbidity of PTSD and immune system dysfunction: opportunities for treatment. *Curr Opin Pharmacol*. 2017;29:104-110. doi:10.1016/j.coph.2016.07.011.Co-Morbidity
141. Almouzni G, Cedar H. Maintenance of epigenetic information. *Cold Spring Harb Perspect Biol*. 2016;8:a019372. doi:10.1101/cshperspect.a019372
142. Goldberg AD, Allis CD, Bernstein E. Epigenetics: A Landscape Takes Shape. *Cell*. 2007;128(4):635-638. doi:10.1016/j.cell.2007.02.006
143. Jiang S, Postovit L, Cattaneo A, Binder EB, Aitchison KJ. Epigenetic Modifications in Stress Response Genes Associated With Childhood Trauma. *Front Psychiatry*. 2019;10(808). doi:10.3389/fpsyt.2019.00808
144. Antequera F, Bird A. CpG islands as genomic footprints of promoters that are

associated with replication origins. *Curr Biol*. 1999;9(17):R661-R667. doi:10.1016/S0960-9822(99)80418-7

145. Ehrlich M, Lacey M. DNA methylation and differentiation: silencing, upregulation and modulation of gene expression. *Epigenomics*. 2013;5(5):553-568. doi:10.2217/epi.13.43
146. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet*. 2012;13(7):484-492. doi:10.1038/nrg3230
147. Mukherjee K, Twyman RM, Vilcinskas A. Insects as models to study the epigenetic basis of disease Insects as models to study the epigenetic basis of disease. *Prog Biophys Mol Biol*. 2015;118(1-2):69-78. doi:10.1016/j.pbiomolbio.2015.02.009
148. Klengel T, Pape J, Binder EB, Mehta D. The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology*. 2014;80:115-132. doi:10.1016/j.neuropharm.2014.01.013
149. Zannas AS, Provençal N, Binder EB. Review Epigenetics of Posttraumatic Stress Disorder : Current Evidence , Challenges , and Future Directions. *Biol Psychiatry*. 2015;78(5):327-335. doi:10.1016/j.biopsych.2015.04.003
150. Wilkening S, Chen B, Bermejo JL, Canzian F. Is there still a need for candidate gene approaches in the era of genome-wide association studies? *Genomics*. 2009;93(5):415-419. doi:10.1016/j.ygeno.2008.12.011
151. Cornelis MC, Nugent NR, Amstadter AB, Koenen KC. Genetics of post-traumatic stress disorder: Review and recommendations for genome-wide association studies. *Curr Psychiatry Rep*. 2010;12(4):313-326. doi:10.1007/s11920-010-0126-6
152. Assary E, Vincent JP, Keers R, Pluess M. Seminars in Cell & Developmental Biology Gene-environment interaction and psychiatric disorders : Review and future directions. *Semin Cell Dev Biol*. 2018;77:133-143. doi:10.1016/j.semcdb.2017.10.016
153. Duncan L, Matthew C. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatry*. 2011;10:1041-1049.
154. Broekman BFP, Olff M, Boer F. The genetic background to PTSD. *Neurosci Biobehav Rev*. 2007;31(3):348-362. doi:10.1016/j.neubiorev.2006.10.001
155. Alghamdi J, Padmanabhan S. Fundementals of Complex Trait Genetics and Association Studies. In: Padmanabhan S, ed. *Handbook of Pharmacogenomics and Stratified Medicine*. Glasgow, United Kingdom: Academic Press; 2014:235-257. doi:10.1016/B978-0-12-386882-4.00012-8
156. Uitterlinden A. Genetics of the Vitamin D Endocrine System. In: Feldman D, Wesley

P, Adams J, eds. *Vitamin D*. 3rd ed. Rotterdam, The Netherlands: Academic Press; 2011:1025-1039. doi:10.1016/B978-0-12-381978-9.10056-3

157. Hansen K, Langmead B, Irizarry R. BSmooth: from whole genome bisulfite sequencing reads to differentially methylated regions. *Genome Biol*. 2012;13(10).
158. Sun Z, Cunningham J, Slager S, Kocher J. Base resolution methylome profiling: considerations in platform selection, data preprocessing and analysis. *Epigenomics*. 2015;7(5):813-828. doi:https://doi.org/10.2217/epi.15.21
159. Kraft P, Zeggini E, Loannidis J. Replication in genome-wide association studies. *Stat Sci*. 2009;24(4):561–573.
160. Smith A, Ratanatharathorn A, Maihofer A, et al. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies novel methylation loci. *bioRxiv Prepr*. 2019. doi:https://doi.org/10.1101/585109
161. Logue MW, Miller MW, Wolf EJ, et al. An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clin Epigenetics*. 2020;12(1):1-14. doi:10.1186/s13148-020-0820-0
162. Snijders C, Maihofer AX, Ratanatharathorn A, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clin Epigenetics*. 2020;12(1):1-13. doi:10.1186/s13148-019-0798-7
163. Rutten BPF, Vermetten E, Vinkers CH, et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry*. 2018;23(5):1145-1156. doi:10.1038/mp.2017.120
164. Mehta D, Bruenig D, Carrillo-Roa T, et al. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatr Scand*. 2017;136(5):493-505. doi:10.1111/acps.12778
165. Hammamieh R, Chakraborty N, Gautam A, et al. Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Transl Psychiatry*. 2017;7(7):e1169. doi:10.1038/tp.2017.129
166. Kuan PF, Waszczuk MA, Kotov R, et al. An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Transl Psychiatry*. 2017;7(6):e1158. doi:10.1038/tp.2017.130
167. Uddin M, Aiello AE, Wildman DE, et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A*.

2010;107(20):9470-9475. doi:10.1073/pnas.0910794107

168. Smith A, Conneely K, Kilaru V, et al. Differential immune system DNA methylation and cytokine regulation in Post-Traumatic Stress Disorder. *Am J Med Genet Paert B Neuropsychiatr Genet*. 2011;156B(6):700-708. doi:10.1109/TMI.2012.2196707.Separate
169. Mehta D, Klengel T, Conneely KN, et al. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2013;110(20):8302-8307. doi:10.1073/pnas.1217750110
170. Chen Y, Li X, Kobayashi I, Tsao D, Mellman T. Expression and methylation in Posttraumatic Stress Disorder and resilience; evidence of the role of odorant receptors. *Psychiatry Res*. 2016;245:36-44. doi:10.1016/j.physbeh.2017.03.040
171. Krzyzewska IM, Ensink JBM, Nawijn L, et al. Genetic variant in CACNA1C is associated with PTSD in traumatized police officers. *Eur J Hum Genet*. 2018;26(2):247-257. doi:10.1038/s41431-017-0059-1
172. Maddox SA, Kilaru V, Shin J, et al. Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. *Mol Psychiatry*. 2018;23(3):658-665. doi:10.1038/mp.2016.250
173. Uddin M, Ratanatharathorn A, Armstrong D, Kuan P, Allison E. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics*. 2018;10(12):1585-1601.
174. Ressler K, Mercer K, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and PAC1 receptor. *Nature*. 2011;470(7335):492-497. doi:10.1097/CCM.0b013e31823da96d.Hydrogen
175. Miller MW, Maniates H, Wolf EJ, et al. Brain , Behavior , and Immunity CRP polymorphisms and DNA methylation of the AIM2 gene influence associations between trauma exposure , PTSD , and C-reactive protein. *Brain Behav Immun*. 2018;67:194-202. doi:10.1016/j.bbi.2017.08.022
176. Moser DA, Paoloni-Giacobino A, Stenz L, et al. BDNF methylation and maternal brain activity in a violence-related sample. *PLoS One*. 2015;10(12):e0143427. doi:10.1371/journal.pone.0143427
177. Kim TY, Kim SJ, Chung HG, Choi JH, Kim SH, Kang JI. Epigenetic alterations of the BDNF gene in combat-related post-traumatic stress disorder. *Acta Psychiatr Scand*. 2017;135(2):170-179. doi:10.1111/acps.12675
178. Norrholm SD, Jovanovic T, Smith AK, et al. Differential genetic and epigenetic

regulation of catechol-O-methyl-transferase (COMT) is associated with impaired fear inhibition in posttraumatic stress disorder. *Front Behav Neurosci*. 2013;7(MAR):1-10. doi:10.3389/fnbeh.2013.00030

179. Bishop JR, Lee AM, Mills LJ, et al. Methylation of FKBP5 and SLC6A4 in Relation to Treatment Response to Mindfulness Based Stress Reduction for Posttraumatic Stress Disorder. *Front Psychiatry*. 2018;9(September):1-11. doi:10.3389/fpsyt.2018.00418
180. Yehuda R, Daskalakis NP, Bierer LM, et al. Archival Report Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. *Biol Psychiatry*. 2016;80(5):372-380. doi:10.1016/j.biopsych.2015.08.005
181. Kang J, Kim T, Choi J, So H, Kim S. Allele-specific DNA methylation level of FKBP5 is associated with post- traumatic stress disorder. *Psychoneuroendocrinology*. 2019;103(November 2018):1-7. doi:10.1016/j.psyneuen.2018.12.226
182. Yehuda R, Nikolaos P, Koch E, et al. Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. *Front Psychiatry*. 2013;4(September):1-14. doi:10.3389/fpsyt.2013.00118
183. Schechter DS, Moser DA, Pointet VC, et al. The association of serotonin receptor 3A methylation with maternal violence exposure, neural activity, and child aggression. *Behav Brain Res*. 2017;325:268-277. doi:10.1016/j.bbr.2016.10.009
184. Bam M, Yang X, Zhou J, Ginsberg JP, Leyden Q. Evidence for Epigenetic Regulation of Pro-Inflammatory Cytokines , Interleukin-12 and Interferon Gamma , in Peripheral Blood Mononuclear Cells from PTSD Patients. *J Neuroimmune Pharmacol*. 2016;11:168-181. doi:10.1007/s11481-015-9643-8
185. Uddin M, Galea S, Chang S, Aiello AE, Wildman DE. Gene expression and methylation signatures of MAN2C1 are associated with PTSD. *Dis Markers*. 2011;30:111-121. doi:10.3233/DMA-2011-0750
186. Ziegler C, Wolf C, Schiele MA, et al. Monoamine Oxidase A Gene Methylation and Its Role in Posttraumatic Stress Disorder: First Evidence from the South Eastern Europe (SEE)-PTSD Study. *Int J Neuropsychopharmacol*. 2018;21(5):423-432. doi:10.1093/ijnp/pyx111
187. Labonté B, Azoulay N, Yerko V, Turecki G, Brunet A. Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder. 2014;(November 2013). doi:10.1038/tp.2014.3
188. Schechter D, Moser D, Paoloni-Giacobino A, et al. Methylation of NR3C1 is related to maternal PTSD , parenting stress and maternal medial prefrontal cortical activity in

response to child separation among mothers with histories of violence exposure. *Front Psychol*. 2015;6(May):1-12. doi:10.3389/fpsyg.2015.00690

189. Schür RR, Boks MP, Rutten BPF, et al. Longitudinal changes in glucocorticoid receptor exon 1F methylation and psychopathology after military deployment. *Transl Psychiatry*. 2017;7:e1181. doi:10.1038/tp.2017.150
190. Vukojevic V, Kolassa I, Fastenrath M, et al. Epigenetic Modification of the Glucocorticoid Receptor Gene Is Linked to Traumatic Memory and Post-Traumatic Stress Disorder Risk in Genocide Survivors. *J Neurosci*. 2014;34(31):10274-10284. doi:10.1523/JNEUROSCI.1526-14.2014
191. Yehuda R, Flory JD, Bierer LM, et al. Lower Methylation of Glucocorticoid Receptor Gene Promoter 1 F in Peripheral Blood of Veterans with Posttraumatic Stress Disorder. *Biol Psychiatry*. 2015;77(4):356-364. doi:10.1016/j.biopsych.2014.02.006
192. Mcnerney MW, Sheng T, Nechvatal JM, et al. Integration of neural and epigenetic contributions to posttraumatic stress symptoms : The role of hippocampal volume and glucocorticoid receptor gene methylation. *PLoS One*. 2018;13(2):1-14.
193. Nawijn L, van Zuiden M, Frijling JL, Koch SBJ, Veltman DJ, Olff M. Reward functioning in PTSD: A systematic review exploring the mechanisms underlying anhedonia. *Neurosci Biobehav Rev*. 2015;51:189-204. doi:10.1016/j.neubiorev.2015.01.019
194. Sadeh N, Spielberg J, Logue M, et al. SKA2 Methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed veterans. *Mol Psychiatry*. 2016;21(3):357-363. doi:10.1038/mp.2015.134.SKA2
195. Boks MP, Rutten BPF, Geuze E, et al. SKA2 Methylation is Involved in Cortisol Stress Reactivity and Predicts the Development of Post-Traumatic Stress Disorder (PTSD) After Military Deployment. *Neuropsychopharmacoly*. 2016;41:1350-1356. doi:10.1038/npp.2015.286
196. Chang SC, Koenen KC, Galea S, et al. Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. *PLoS One*. 2012;7(6):1-6. doi:10.1371/journal.pone.0039184
197. Koenen K, Uddin M, Chang S, et al. SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. *Depress Anxiety*. 2011;28:639-647. doi:10.1002/da.20825
198. Sherin JE, Nemeroff CB. Post-traumatic stress disorder: the neurobiological impact of psychological trauma. *Dialogues Clin Neurosci*. 2011;13:263-278.

199. Newport DJ, Nemeroff CB. Neurobiology of posttraumatic stress disorder. *Cogn Neurosci*. 2000;10(2):211-218.

200. Ross D, Arbuckle M, Travis M, Dwyer J, van Schalkwyk G, Ressler K. An Integrated Neuroscience Perspective on Formulation and Treatment Planning for Posttraumatic Stress Disorder An Educational Review. *JAMA Psychiatry*. 2017;74(4):407-415. doi:10.1001/jamapsychiatry.2016.3325.An

201. Suvak MK, Barrett LF. Considering PTSD From the Perspective of Brain Processes : A Psychological Construction Approach. *J Trauma Stress*. 2011;24(1):3-24. doi:10.1002/jts.

202. Liberzon I, Abelson JL. Perspective Context Processing and the Neurobiology of Post-Traumatic Stress Disorder. *Neuron*. 2016;92(1):14-30. doi:10.1016/j.neuron.2016.09.039

203. Smith N, Doran J, Sippel L, Harpaz-Rotem I. Fear extinction and memory reconsolidation as critical components in behavioral treatment for posttraumatic stress disorder and potential augmentation of these processes. *Neurosci Lett*. 2017;649:170-175.

204. Sadeghi R, Mokhber N, Mahmoudi L, Asgharipour N, Seyfi H. A systematic review and meta-analysis on controlled treatment trials of metacognitive therapy for anxiety disoders. *J Res Med Sci*. 2015;20(9):901-909.

205. Bonne O, Vythilingam M, Inagaki M, et al. Reduced posterior hippocampal volume in posttraumatic stress disorder. *J Clin Psychiatry*. 2008;69(7):1087-1091.

206. Bremner JD, Randall P, Scott TM, et al. MRI-Based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Am J Psychiatry*. 1995;152(7):973-981.

207. Chao LL, Yaffe K, Samuelson K, Neylan TC. Psychiatry Research : Neuroimaging Hippocampal volume is inversely related to PTSD duration. *Psychiatry Res Neuroimaging*. 2014;i:119-123.

208. Bremner JD, Randall P, Vermetten E, et al. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse - A preliminary report. *Biol Psychiatry*. 1997;41(1):23-32.

209. Gurvits T V, Shenton ME, Hokama H, et al. Magnetic resonance imaging study of hippocampal volume in chronis, combat-related posttraumatic stress disorder. *Biol Psychiatry*. 1996;40(11):1091-1099. doi:10.1016/S0006-3223(96)00229-6.Magnetic

210. Moyer A. Post-traumatic Stress Disorder and Magnetic Resonance Imaging. 2016;87(6):649-669.
211. Longue MW, van Rooij SJH, Morey RA. Smaller hippocampal volume in posttraumatic stress disorder: a multisite ENIGMA-PGC study: subcortical volumetry results from posttraumatic stress disorder consortia. *Biol Psychiatry*. 2018;83(3):244-253. doi:10.1016/j.biopsych.2017.09.006.Smaller
212. Kuhn S, Gallinat J. Gray matter correlates of posttraumatic stress disorder: A quantitative meta-analysis. *Biol Psychiatry*. 2013;1:70-74.
213. Henigsberg N, Kalember P, Kova Z, Ana Š. Progress in Neuropsychopharmacology & Biological Psychiatry Neuroimaging research in posttraumatic stress disorder – Focus on amygdala , hippocampus and prefrontal cortex. 2019;90(November 2018):37-42. doi:10.1016/j.pnpbp.2018.11.003
214. Garfinkel SN, Abelson JL, King AP, et al. Impaired Contextual Modulation of Memories in PTSD : An fMRI and Psychophysiological Study of Extinction Retention and Fear Renewal. *Neuroniology Dis*. 2014;34(40):13435-13443. doi:10.1523/JNEUROSCI.4287-13.2014
215. Milad MR, Pitman RK, Ellis CB, et al. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry*. 2010;66(12):1075-1082. doi:10.1016/j.biopsych.2009.06.026.Neurobiological
216. Rougemont-Bucking A, Linnman C, Zeffiro TA, et al. Altered Processing of Contextual Information during Fear Extinction in PTSD : An fMRI Study. *Neurosci Ther*. 2011;17:227-236. doi:10.1111/j.1755-5949.2010.00152.x
217. Stevens JS, Reddy R, Ji Y, et al. NeuroImage : Clinical Episodic memory after trauma exposure : Medial temporal lobe function is positively related to re-experiencing and inversely related to negative a ff ect symptoms. *NeuroImage Clin*. 2018;17(November 2017):650-658. doi:10.1016/j.nicl.2017.11.016
218. Rauch SL, Shin LM, Phelps EA. Neurocircuitry Models of Posttraumatic Stress Disorder and Extinction: Human Neuroimaging Research—Past, Present, and Future. *Biol Psychiatry*. 2006;60(4):376-382. doi:10.1016/j.biopsych.2006.06.004
219. Shin LM, Rauch SL, Pitman RK. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann N Y Acad Sci*. 2006;1071:67-79. doi:10.1196/annals.1364.007
220. Baldaçara L, Zugman A, Araújo C, et al. Reduction of anterior cingulate in adults with urban violence- Related PTSD. *J Affect Disord*. 2014;168:13-20.

doi:10.1016/j.jad.2014.06.036

221. Kuo JR, Kaloupek DG, Woodward SH. Amygdala volume in combat-exposed veterans with and without posttraumatic stress disorder: A cross-sectional study. *Arch Gen Psychiatry*. 2012;69(10):1080-1086. doi:10.1001/archgenpsychiatry.2012.73
222. Gianaros PJ, Sheu LK, Matthews KA, Jennings RR, Manuck SB, Hariri AR. Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *J Neurosci*. 2008;28(4):990-999. doi:10.1523/JNEUROSCI.3606-07.2008
223. Morey RA, Gold AL, LaBar KS, et al. Amygdala volume changes in posttraumatic stress disorder in a large case-controlled veterans group. *Arch Gen Psychiatry*. 2012;69(11):1169-1178. doi:10.1001/archgenpsychiatry.2012.50
224. Yang RJ, Mozhui K, Karlsson R-M, Cameron HA, Williams RW, Holmes A. Variation in Mouse Basolateral Amygdala Volume is Associated With Differences in Stress Reactivity and Fear Learning. *Neuropsychopharmacology*. 2008;33(11):2595-2604. doi:10.1038/sj.npp.1301665
225. Driessen M, Beblo T, Mertens M, et al. Posttraumatic stress disorder and fMRI activation patterns of traumatic memory in patients with borderline personality disorder. *Biol Psychiatry*. 2004;55(6):603-611. doi:10.1016/j.biopsych.2003.08.018
226. Jovanovic T, Kazama A, Bachevalier J. Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology*. 2013;62(2):695-704. doi:10.1016/j.neuropharm.2011.02.023.Impaired
227. Liberzon I, Taylor SF, Amdur R, et al. Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiatry*. 1999;45(7):817-826. doi:10.1016/S0006-3223(98)00246-7
228. Shin LM, Wright CI, Cannistraro PA, et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry*. 2005;62(3):273-281. doi:10.1001/archpsyc.62.3.273
229. Shin LM, Orr SP, Carson MA, et al. Regional Cerebral Blood Flow in the Amygdala and Medial Prefrontal Cortex during Traumatic Imagery in Male and Female Vietnam Veterans with PTSD. *Arch Gen Psychiatry*. 2004;61(2):168-176. doi:10.1001/archpsyc.61.2.168
230. Fitzgerald JM, Digangi JA, Phan KL, Biology C. Functional neuroanatomy of emotion and its regulation in PTSD. *Harvard Rev Psychiatry*. 2019;26(3):116-128.

doi:10.1097/HRP.0000000000000185.Functional

231. Mcewen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*. 2016:3-23. doi:10.1038/npp.2015.171
232. Carrion VG, Weems CF, Eliez S, et al. Attenuation of frontal asymmetry in pediatric posttraumatic stress disorder. *Biol Psychiatry*. 2001;50(12):943-951. doi:10.1016/S0006-3223(01)01218-5
233. Fennema-Notestine C, Stein MB, Kennedy CM, Archibald SL, Jernigan TL. Brain morphometry in female victims of intimate partner violence with and without posttraumatic stress disorder. *Biol Psychiatry*. 2002;52(11):1089-1101. doi:10.1016/S0006-3223(02)01413-0
234. Kasai K, Yamasue H, Gilbertson MW, Shenton ME, Rauch SL, Pitman RK. Evidence for Acquired Pregenual Anterior Cingulate Gray Matter Loss from a Twin Study of Combat-Related Posttraumatic Stress Disorder. *Biol Psychiatry*. 2008;63(6):550-556. doi:10.1016/j.biopsych.2007.06.022
235. Li L, Wu M, Liao Y, et al. Grey matter reduction associated with posttraumatic stress disorder and traumatic stress. *Neurosci Biobehav Rev*. 2014;43(37):163-172. doi:10.1016/j.neubiorev.2014.04.003
236. Brenner LA. Neuropsychological and neuroimaging findings in traumatic brain injury and post-traumatic stress disorder. *Dialogues Clin Neurosci*. 2011;13(3):311-323.
237. Etkin A, Wager TD. Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. *Am J Psychiatry*. 2007;164(10):1476-1488. doi:10.1176/appi.ajp.2007.07030504
238. Hughes K SL. Function neuroimaging studies of PTSD. *Expert Rev Neurother*. 2011;11(2):275-285. doi:10.1586/ern.10.198.Functional
239. Lanius RA, Williamson PC, Densmore M, et al. Neural Correlates of Traumatic Memories in Posttraumatic Stress Disorder: A Functional MRI Investigation. *Am J Psychiatry*. 2001;158(11):1920-1922. doi:10.1176/appi.ajp.158.11.1920
240. Lanius RA, Williamson PC, Hopper J, et al. Recall of emotional states in posttraumatic stress disorder: An fMRI investigation. *Biol Psychiatry*. 2003;53(3):204-210. doi:10.1016/S0006-3223(02)01466-X
241. Szeszko PR, Lehrner A, Yehuda R. Glucocorticoids and Hippocampal Structure and Function in PTSD. *Harv Rev Psychiatry*. 2018;26(3):142-157. doi:10.1097/HRP.0000000000000188

242. Moser DA, Paoloni-Giacobino A, Stenz L, et al. BDNF methylation and maternal brain activity in a violence-related sample. *PLoS One*. 2015;10(12):1-13. doi:10.1371/journal.pone.0143427

243. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci*. 2015:1164-1178. doi:10.5114/aoms.2015.56342

244. Dell'Osso L, Carmassi C, Del Debbio A, et al. Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic stress disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2009;33(5):899-902. doi:10.1016/j.pnpbp.2009.04.018

245. Maina G, Rosso G, Zanardini R, Bogetto F, Gennarelli M, Bocchio-Chiavetto L. Serum levels of brain-derived neurotrophic factor in drug-naïve obsessive–compulsive patients: A case–control study. *J Affect Disord*. 2010;122(1-2):174-178. doi:10.1016/j.jad.2009.07.009

246. Wang Y, Mathews CA, Li Y, Lin Z, Xiao Z. Brain-derived neurotrophic factor (BDNF) plasma levels in drug-naïve OCD patients are lower than those in healthy people, but are not lower than those in drug-treated OCD patients. *J Affect Disord*. 2011;133(1-2):305-310. doi:10.1016/j.jad.2011.04.002

247. dos Santos IM, Ciulla L, Braga D, et al. Symptom Dimensional Approach and BDNF in Unmedicated Obsessive-Compulsive Patients: An Exploratory Study . *CNS Spectr*. 2011;16(9):179-189. doi:10.1017/s1092852912000363

248. Ströhle A, Stoy M, Graetz B, et al. Acute exercise ameliorates reduced brain-derived neurotrophic factor in patients with panic disorder. *Psychoneuroendocrinology*. 2010;35(3):364-368. doi:10.1016/j.psyneuen.2009.07.013

249. Suliman S, Hemmings SMJ, Seedat S. Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders : systematic review and meta-regression analysis. *Front Integr Neurosci*. 2013;7(July):1-11. doi:10.3389/fnint.2013.00055

250. Notaras M, van den Buuse M. Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. *Mol Psychiatry*. 2020;25:2251-2274. doi:10.1038/s41380-019-0639-2

251. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*. 2003;112(2):257-269. doi:10.1016/S0092-8674(03)00035-7

252. Bruenig D, Lurie J, Morris CP, et al. A case-control study and meta-analysis reveal BDNF Val66Met is a possible risk factor for PTSD. *Neural Plast*. 2016:6979435.

doi:10.1155/2016/6979435

253. Felmingham KL, Dobson-Stone C, Schofield PR, Quirk GJ, Bryant RA. The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. *Biol Psychiatry*. 2013;73(11):1059-1063. doi:10.1016/j.biopsych.2012.10.033

254. Guo JC, Yang YJ, Zheng JF, et al. Functional rs6265 polymorphism in the brain-derived neurotrophic factor gene confers protection against neurocognitive dysfunction in posttraumatic stress disorder among Chinese patients with hepatocellular carcinoma. *J Cell Biochem*. 2019;120(6):10434-10443. doi:10.1002/jcb.28328

255. Li RH, Fan M, Hu MS, Ran MS, Fang DZ. Reduced severity of posttraumatic stress disorder associated with Val allele of Val66Met polymorphism at brain-derived neurotrophic factor gene among Chinese adolescents after Wenchuan earthquake. *Psychophysiology*. 2016;53(5):705-711. doi:10.1111/psyp.12603

256. Pitts BL, Whealin JM, Harpaz-Rotem I, et al. BDNF Val66Met polymorphism and posttraumatic stress symptoms in U.S. military veterans: Protective effect of physical exercise. *Psychoneuroendocrinology*. 2019;100(August 2018):198-202. doi:10.1016/j.psyneuen.2018.10.011

257. Pivac N, Kozaric-Kovacic D, Grubisic-Ilic M, et al. The association between brain-derived neurotrophic factor Val66Met variants and psychotic symptoms in posttraumatic stress disorder. *World J Biol Psychiatry*. 2012;13(4):306-311. doi:10.3109/15622975.2011.582883

258. Leal G, Afonso PM, Salazar IL, Duarte CB. Regulation of hippocampal synaptic plasticity by BDNF. *Brain Res*. 2015;1621:82-101. doi:10.1016/j.brainres.2014.10.019

259. Bremner J. Stress and Brain Atrophy. *CNS Neurol Disord Drug Targets*. 2006;5(5):503-512. doi:10.1038/jid.2014.371

260. Notaras M, Hill R, Van den Buuse M. A role for the BDNF gene Val66Met polymorphism in schizophrenia? A comprehensive review. *Neurosci Biobehav Rev*. 2015;51:15-30. doi:10.1016/j.neubiorev.2014.12.016

261. Notaras M, Hill R, Van Den Buuse M. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: Progress and controversy. *Mol Psychiatry*. 2015;20(8):916-930. doi:10.1038/mp.2015.27

262. Chen ZY, Jing D, Bath KG, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science (80-)*. 2006;314(5796):140-143. doi:10.1126/science.1129663

263. Enciu AM, Nicolescu MI, Manole CG, Mureşanu DF, Popescu LM, Popescu BO. Neuroregeneration in neurodegenerative disorders. *BMC Neurol*. 2011;11(75):1-7. doi:10.1186/1471-2377-11-75

264. Wiesmann C, De Vos AM. Nerve growth factor: Structure and function. *Cell Mol Life Sci*. 2001;58(5-6):748-759. doi:10.1007/PL00000898

265. Fumagalli F, Molteni R, Calabrese F, Maj PF, Racagni G, Riva MA. Neurotrophic factors in neurodegenerative disorders: Potential for therapy. *CNS Drugs*. 2008;22(12):1005-1019. doi:10.2165/0023210-200822120-00004

266. Seilheimer B, Schachner M. Studies of adhesion molecules mediating interactions between cells of peripheral nervous system indicate a major role for L1 in mediating sensory neuron growth on Schwann cells in culture. *J Cell Biol*. 1988;107(1):341-351. doi:10.1083/jcb.107.1.341

267. Araki T, Milbrandt J. Ninjurin2, a novel homophilic adhesion molecule, is expressed in mature sensory and enteric neurons and promotes neurite outgrowth. *J Neurosci*. 2000;20(1):187-195. doi:10.1523/jneurosci.20-01-00187.2000

268. Burgoon M, Hazan R, Phillips G, Crossin K, Edelman G, Cunnungham B. Functional analysis of posttranslational cleavage products of the neuron-glia cell adhesion molecule, Ng-CAM. *J Cell Biol*. 1995;130(3):733-744.

269. Dimou L, Schnell L, Montani L, et al. Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. *J Neurosci*. 2006;26(21):5591-5603. doi:10.1523/JNEUROSCI.1103-06.2006

270. Li W, Wang X, Zhao J, et al. Association study of myelin transcription factor 1-like polymorphisms with schizophrenia in Han Chinese population. *Genes, Brain Behav*. 2012;11(1):87-93. doi:10.1111/j.1601-183X.2011.00734.x

271. Teschler S, Bartkuhn M, Künzel N, et al. Aberrant methylation of gene associated CpG sites occurs in borderline personality disorder. *PLoS One*. 2013;8(12):e84180. doi:10.1371/journal.pone.0084180

272. Nielsen JA, Berndt JA, Hudson LD, Armstrong RC. Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells. *Mol Cell Neurosci*. 2004;25(1):111-123. doi:10.1016/j.mcn.2003.10.001

273. Romm E, Nielsen JA, Kim JG, Hudson LD. Myt1 family recruits histone deacetylase to regulate neural transcription. *J Neurochem*. 2005;93(6):1444-1453. doi:10.1111/j.1471-4159.2005.03131.x

274. Ploski JE, Park KW, Ping J, Monsey MS, Schafe GE. Identification of plasticity-

associated genes regulated by Pavlovian fear conditioning in the lateral amygdala. *J Neurochem*. 2010;112(3):636-650. doi:10.1111/j.1471-4159.2009.06491.x

275. Datson NA, Van Den Oever JME, Korobko OB, Magarinos AM, De Kloet ER, McEwen BS. Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. *Endocrinology*. 2013;154(9):3261-3272. doi:10.1210/en.2012-2233
276. Wang Z, Neylan TC, Mueller SG, et al. Magnetic Resonance Imaging of Hippocampal Subfields in Posttraumatic Stress Disorder. *Arch Gen Psychiatry*. 2010;67(3):296-303.
277. Friedman MJ, Wang S, Jalowiec JE, McHugo GJ, McDonagh-Coyle A. Thyroid Hormone Alterations Among Women with Posttraumatic Stress Disorder Due to Childhood Sexual Abuse. *Biol Psychiatry*. 2005;57(10):1186-1192. doi:10.1016/j.biopsych.2005.01.019
278. Siegmund KD, Connor CM, Campan M, et al. DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. *PLoS One*. 2007;2(9). doi:10.1371/journal.pone.0000895
279. Nisar S, Bhat AA, Hashem S, et al. Genetic and neuroimaging approaches to understanding post-traumatic stress disorder. *Int J Mol Sci*. 2020;21(12):1-21. doi:10.3390/ijms21124503
280. Kim JSM, Lilley BN, Zhang C, Shokat KM, Sanes JR, Zhen M. A chemical-genetic strategy reveals distinct temporal requirements for SAD-1 kinase in neuronal polarization and synapse formation. *Neural Dev*. 2008;3(23):1-14. doi:10.1186/1749-8104-3-23
281. Sample V, Ramamurthy S, Gorshkov K, Ronnett G V, Chernoff J. Polarized activities of AMPK and BRSK in primary hippocampal neurons. 2015;26:1935-1946. doi:10.1091/mbc.E14-02-0764
282. Krystal J, Neumeister A. Noradrenergic and serotonergic mechanisms in the neurobiology of posttraumatic stress disorder and resilience. *Brain Res*. 2009;1293:13-23. doi:10.1016/j.brainres.2009.03.044.Noradrenergic
283. Kelmendi B, Adams TG, Yarnell S, Southwick S, Abdallah CG, Krystal JH. PTSD: from neurobiology to pharmacological treatments. *Eur J Psychotraumatol*. 2016;7(1):31858. doi:10.3402/ejpt.v7.31858
284. Goldstein D. Adrenal responses to stress. *Cell Mol Neurobiol*. 2010;30:1433-1440.
285. Geracioti T, Baker D, Ekhator N, et al. CSF Norepinephrine concentrations in Posttraumatic Stress Disorder. *Am J Psychiatry*. 2001;158:1227-1230.

286. Delahanty DL, Nugent NR, Christopher NC, Walsh M. Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendrocrinology*. 2005;30:121-128. doi:10.1016/j.psyneuen.2004.06.004

287. Kaur S, Singh R. Role of different neurotransmitters in anxiety: a systemic review. *Int J Pharm Sci Res*. 2017;8(2):411-421. doi:10.13040/IJPSR.0975-8232.8(2).411-21

288. Chen J, Lipska BK, Halim N, et al. Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589

289. Almli LM, Fani N, Smith AK, Ressler KJ. Genetic approaches to understanding post-traumatic stress disorder. *Int J Neuropsychopharmacol*. 2014;17(02):355-370. doi:10.1017/S1461145713001090

290. Drabant EM, Hariri AR, Meyer-Lindenberg A, et al. Catechol O-methyltransferase Val158Met Genotype and Neural Mechanisms Related to Affective Arousal and Regulation. *Arch Gen Psychiatry*. 2006;63(12):1396-1406. doi:10.1001/archpsyc.63.12.1396

291. Massat I, Souery D, Del-Favero J, et al. Association between COMT (Val158Met) functional polymorphism and early onset in patients with major depressive disorder in a European multicenter genetic association study. *Mol Psychiatry*. 2005;10(6):598-605. doi:10.1038/sj.mp.4001615

292. Weiss SJ. Neurobiological alteration associated with traumatic stress. *Perspect Psychiatr Care*. 2007;43(3):114-122.

293. Hamner MB, Diamond BI. Elevated plasma dopamine in posttraumatic stress disorder: A preliminary report. *Biol Psychiatry*. 1993;33(4):304-306. doi:10.1016/0006-3223(93)90302-T

294. Yehuda R, Southwick S, GIller E, Xiaowan N, Mason J. Urinary Catecholamine Excretion and Severity of PTSD Symptoms in Vietnam Combat Veterans. *J Nerv Ment Dis*. 1992;180(5):321-325. doi:10.1097/00005053-199205000-00006

295. Mill J, Asherson P, Craig I, D'Souza UM. Transient expression analysis of allelic variants of a VNTR in the dopamine transporter gene (DATI). *BMC Genet*. 2005;6(3):1-7. doi:10.1186/1471-2156-6-3

296. Shumay E, Fowler JS, Volkow ND. Genomic features of the human dopamine transporter gene and its potential epigenetic states: Implications for phenotypic diversity. *PLoS One*. 2010;5(6):e11067. doi:10.1371/journal.pone.0011067

297. Vermetten E, Vythilingam M, Southwick S, Charney D, Bremner J. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. *Biol Psychiatry*. 2003;54(7):693-702.

298. Seedat S, Warwick J, van Heerden B, et al. Single photon emission computed tomography in posttraumatic stress disorder before and after treatment with selective serotonin reuptake inhibitor. *J Affect Disord*. 2004;80(1):45-53. doi:10.1016/S0165-0327(03)00047

299. Arce E, Simmons AN, Lovero KL, Stein MB, Paulus MP. Escitalopram effects on insula and amygdala BOLD activation during emotional processing. *Psychopharmacology (Berl)*. 2008;196:661-672. doi:10.1007/s00213-007-1004-8

300. Gatt JM, Nemeroff CB, Schofield PR, et al. Early Life Stress Combined with Serotonin 3A Receptor and Brain-Derived Neurotrophic Factor Valine 66 to Methionine Genotypes Impacts Emotional Brain and Arousal Correlates of Risk for Depression. *Biol Psychiatry*. 2010;68(9):818-824. doi:10.1016/j.biopsych.2010.06.025

301. Jang K-I, Lee S-H, Huh HJ, Chae J-H. Influence of the 5-HT3A Receptor Gene Polymorphism and Childhood Sexual Trauma on Central Serotonin Activity. Homberg J, ed. *PLoS One*. 2015;10(12):e0145269. doi:10.1371/journal.pone.0145269

302. Hammer C, Cichon S, Mühleisen TW, et al. Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study. *Transl Psychiatry*. 2012;2(4):e103-e103. doi:10.1038/tp.2012.30

303. Niesler B, Flohr T, Nothen M, et al. Association between the 5'UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. *Pharmacogenet Genomics*. 2001;11(6):471-475.

304. Perroud N, Zewdie S, Stenz L, et al. METHYLATION OF SEROTONIN RECEPTOR 3A IN ADHD, BORDERLINE PERSONALITY, AND BIPOLAR DISORDERS: LINK WITH SEVERITY OF THE DISORDERS AND CHILDHOOD MALTREATMENT. *Depress Anxiety*. 2016;33(1):45-55. doi:10.1002/da.22406

305. Berumen LC, Rodríguez A, Miledi R, García-Alcocer G. Serotonin Receptors in Hippocampus. *Sci World J*. 2012;2012:1-15. doi:10.1100/2012/823493

306. Yoon S, Kim YK. Neuroendocrinological treatment targets for posttraumatic stress disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;90(June 2018):212-222. doi:10.1016/j.pnpbp.2018.11.021

307. Gimpl G, Fahrenholz F. The Oxytocin Receptor System : Structure , Function , and Regulation. *Physiol Rev*. 2001;81(2):629-683.

308. Engel S, Klusmann H, Laufer S, et al. Trauma exposure , posttraumatic stress disorder and oxytocin : A meta- analytic investigation of endogenous concentrations and receptor genotype. *Neurosci Biobehav Rev*. 2019;107(July):560-601. doi:10.1016/j.neubiorev.2019.08.003

309. Matsushita H, Latt HM, Koga Y, Nishiki T, Matsui H. Oxytocin and Stress : Neural Mechanisms , Stress-Related Disorders , and Therapeutic Approaches. *Neurosci Rev*. 2020;417(2019):1-10. doi:10.1016/j.neuroscience.2019.07.046

310. Frijling JL, Zuiden M Van, Nawijn L, et al. Salivary Oxytocin and Vasopressin Levels in Police Officers With and Without Post-Traumatic Stress Disorder Neuroendocrinology. *J Neuroendrocrinology*. 2015;27:743-751. doi:10.1111/jne.12300

311. Koch SBJ, Zuiden M Van, Nawijn L, Frijling JL, Veltman DJ, Olff M. Intranasal Oxytocin Administration Dampens Amygdala Reactivity towards Emotional Faces in Male and Female PTSD Patients. *Neuropsychopharmacology*. 2016;41:1495-1504. doi:10.1038/npp.2015.299

312. Zuiden M Van, Frijling JL, Nawijn L, et al. Intranasal Oxytocin to Prevent Posttraumatic Stress Disorder Symptoms : A Randomized Controlled Trial in Emergency Department Patients. *Biol Psychiatry*. 2017;81(12):1030-1040. doi:10.1016/j.biopsych.2016.11.012

313. Reijnene A, Geuze E, Vermetten E. Individual variation in plasma oxytocin and vasopressin levels in relation to the development of combat-related PTSD in a large military cohort. *J Psychiatr Res*. 2017;94:88-95.

314. Nawijn L, Krzyzewska IM, van Zuiden M, et al. Oxytocin receptor gene methylation in male and female PTSD patients and trauma-exposed controls. *Eur Neuropsychopharmacol*. 2019;29(1):147-155. doi:10.1016/j.euroneuro.2018.10.006

315. Speer KE, Semple S, Naumovski N, D'Cunha NM, McKune AJ. HPA axis function and diurnal cortisol in post-traumatic stress disorder: A systematic review. *Neurobiol Stress*. 2019;11(December 2018):100180. doi:10.1016/j.ynstr.2019.100180

316. Mustafa T. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP). *Adv Pharmacol*. 2013;68:445-457. doi:10.1016/B978-0-12-411512-5.00021-X

317. Lehrer HM, Steinhardt MA, Dubois SK, Laudenslager ML. Psychoneuroendocrinology Perceived stress , psychological resilience , hair cortisol concentration , and metabolic syndrome severity : A moderated mediation model. *Psychoneuroendocrinology*. 2020;113:104510. doi:10.1016/j.psyneuen.2019.104510

318. Zorn J V., Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;77:25-36. doi:10.1016/j.psyneuen.2016.11.036

319. Hammack SE, Cheung J, Rhodes KM, et al. Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): Roles for PACAP in anxiety-like behavior. *Psychoneuroendocrinology*. 2009;34(6):833-843. doi:10.1016/j.psyneuen.2008.12.013

320. Stevens JS, Almli LM, Fani N, et al. PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. *Proc Natl Acad Sci U S A*. 2014;111(8):3158-3163. doi:10.1073/pnas.1318954111

321. Daskalakis NP, Mcgill MA, Lehrner A, Yehuda R. Endocrine aspects of PTSD: hypothalamic-pituitary-adrenal (HPA) axis and beyond. In: Martin C, Preedy V, Patel V, eds. *Comprehensive Guide to Post-Traumatic Stress Disorders*. Switzerland: Springer International Publishing; 2015. doi:10.1007/978-3-319-08359-9

322. Knaap LJ Van Der, Riese H, Hudziak JJ, et al. Glucocorticoid receptor gene (NR3C1) methylation following stressful events between birth and adolescence . The TRAILS study. *Transl Psychiatry*. 2014;4(4):e381-7. doi:10.1038/tp.2014.22

323. Radtke KM, Ruf M, Gunter HM, et al. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Transl Psychiatry*. 2011;1(7):e21-6. doi:10.1038/tp.2011.21

324. Eiden L. Neuropeptide – Catecholamine Interactions in Stress. *Adv Pharmacol*. 2013;68:399-404. doi:10.1016/B978-0-12-411512-5.00018-X.Neuropeptide

325. Pan X, Kaminga AC, Wen SW, Liu A. Catecholamines in Post-traumatic Stress Disorder : A Systematic Review and Meta-Analysis. *Front Mol Neurosci*. 2018;11:1-14. doi:10.3389/fnmol.2018.00450

326. Wang L, Cao C, Wang R, Qing Y, Zhang J. PAC1 receptor (ADCYAP1R1) genotype is associated with PTSD ' s emotional numbing symptoms in Chinese earthquake survivors. *J Affect Disord*. 2013;150(1):156-159. doi:10.1016/j.jad.2013.01.010

327. Ryder AL, Azcarate PM, Cohen BE. PTSD and Physical Health. *Curr Psychiatry Rep*. 2018;20(12). doi:10.1007/s11920-018-0977-9

328. Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. *Nat Rev Immunol*. 2017;17(4):233-247. doi:10.1038/nri.2017.1

329. Rice L, Waters CE, Eccles J, et al. Identification and functional analysis of SKA2

interaction with the glucocorticoid receptor. *J Endocrinol*. 2008;198:499-509. doi:10.1677/JOE-08-0019

330. Pratt WB, Toft DO. Steroid Receptor Interactions with Heat Shock Protein and Immunophilin Chaperones *. *Endrocrine Rev*. 1997;18(3):306-360.
331. Hubler TR, Scammell JG. Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. *Cell Stress Chaperones*. 2004;9(3):243-252. doi:10.1379/CSC-32R.1
332. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*. 2009;34(SUPPL. 1):186-195. doi:10.1016/j.psyneuen.2009.05.021
333. Zannas AS, Wiechmann T, Gassen NC, Binder EB. Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications. *Neuropsychopharmacology*. 2016;41(1):261-274. doi:10.1038/npp.2015.235
334. Szyf M. How do environments talk to genes? *Nat Neurosci*. 2013;16(1):1-4.
335. Jaaskelainen T, Makkonen H, Palvimo J. Steroid up-regulation of FKBP51 and its role in hormone signaling. *Curr Opin Pharmacol*. 2011;11:326-331. doi:10.1016/j.coph.2011.04.006
336. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene – childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41. doi:10.1038/nn.3275
337. Matosin N, Halldorsdottir T, Binder EB. Understanding the Molecular Mechanisms Underpinning Gene by Environment Interactions in Psychiatric Disorders: The FKBP5 Model. *Biol Psychiatry*. 2018;83(10):821-830. doi:10.1016/j.biopsych.2018.01.021
338. Fani N, Gutman D, Tone EB, et al. FKBP5 and attention bias for threat. *JAMA Psychiatry*. 2013;70(4):392-400. doi:10.1001/2013.jamapsychiatry.210
339. Morris MC, Compas BE, Garber J. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: A systematic review and meta-analysis. *Clin Psychol Rev*. 2012;32(4):301-315. doi:10.1016/j.cpr.2012.02.002
340. Meewisse M-L, Reitsma JB, De Vries G-J, Gersons BPR, Olff M. Cortisol and post-traumatic stress disorder in adults. *Br J Psychiatry*. 2007;191(5):387-392. doi:10.1192/bjp.bp.106.024877
341. Kellner M, Yehuda R, Arlt J, Wiedemann K. Case report: Longitudinal course of salivary cortisol in post-traumatic stress disorder. *Acta Psychiatr Scand*.

2002;105(2):153-155. doi:10.1034/j.1600-0447.2002.01012.x

342. de Kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, Westenberg HGM. Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. *J Psychiatr Res*. 2006;40(6):550-567. doi:10.1016/j.jpsychires.2005.08.002
343. Yehuda R, Seckl J. Minireview: Stress-related psychiatric disorders with low cortisol levels: A metabolic hypothesis. *Endocrinology*. 2011;152(12):4496-4503. doi:10.1210/en.2011-1218
344. Mustafa T. Pituitary adenylate cyclase-activating polypeptide (pacap). A master regulator in central and peripheral stress responses. *Adv Pharmacol*. 2013;68:445-457. doi:10.1016/B978-0-12-411512-5.00021-X
345. Boscarino JA. Posttraumatic stress disorder and physical illness: Results from clinical and epidemiologic studies. *Ann N Y Acad Sci*. 2004;1032:141-153. doi:10.1196/annals.1314.011
346. Kubzansky LD, Koenen KC. Is post-traumatic stress disorder related to development of heart disease? *Cleve Clin J Med*. 2009;76(2):60-65. doi:10.2217/14796678.3.2.153
347. Cavalcanti-Ribeiro P, Andrade-Nascimento M, Morais-De-Jesus M, et al. Post-traumatic stress disorder as a comorbidity: Impact on disease outcomes. *Expert Rev Neurother*. 2012;12(8):1023-1037. doi:10.1586/ern.12.77
348. Song H, Fang F, Tomasson G, et al. Association of stress-related disorders with subsequent autoimmune disease. *JAMA - J Am Med Assoc*. 2018;319(23):2388-2400. doi:10.1001/jama.2018.7028
349. Cohen BE, Edmondson D, Kronish IM. State of the art review: Depression, stress, anxiety, and cardiovascular disease. *Am J Hypertens*. 2015;28(11):1295-1302. doi:10.1093/ajh/hpv047
350. Michopoulos V, Vester A, Neigh G. Posttraumatic stress disorder: A metabolic disorder in disguise? *Exp Neurol*. 2016;284:220-229. doi:10.1016/j.expneurol.2016.05.038
351. O'Donovan A, Cohen BE, Seal KH, et al. Elevated risk for autoimmune disorders in iraq and afghanistan veterans with posttraumatic stress disorder. *Biol Psychiatry*. 2015;77(4):365-374. doi:10.1016/j.biopsych.2014.06.015
352. Mellon SH, Gautam A, Hammamieh R, Jett M, Wolkowitz OM. Metabolism, Metabolomics, and Inflammation in Posttraumatic Stress Disorder. *Biol Psychiatry*. 2018;83(10):866-875. doi:10.1016/j.biopsych.2018.02.007

353. Hori H, Kim Y. Inflammation and post-traumatic stress disorder. *Psychiatry Clin Neurosci*. 2019;73:143-153. doi:10.1111/pcn.12820

354. Eraly S, Nievergelt C, Maihofer A, et al. Assessment of Plasma C-Reactive Protein as a Biomarker of Posttraumatic Stress Disorder Risk. *J Am Med Assoc*. 2014;92161(4):423-431. doi:10.1001/jamapsychiatry.2013.4374

355. Kawamura N, Kim Y, Asukai N. Suppression of Cellular Immunity in Men With a Past History of Posttraumatic Stress Disorder. *Am J Psyshiatry*. 2001;158(8):484-486.

356. Gill JM, Saligan L, Lee H, Rotolo S, Szanton S. Women in recovery from PTSD have similar in fl ammation and quality of life as non-traumatized controls. *J Psychosom Res*. 2013;74(4):301-306. doi:10.1016/j.jpsychores.2012.10.013

357. Pace TWW, Heim CM. Brain , Behavior , and Immunity A short review on the psychoneuroimmunology of posttraumatic stress disorder : From risk factors to medical comorbidities. *Brain Behav Immun*. 2011;25(1):6-13. doi:10.1016/j.bbi.2010.10.003

358. Smid GE, van Zuiden M, Geuze E, Kavelaars A, Heijnen CJ, Vermetten E. Cytokine production as a putative biological mechanism underlying stress sensitization in high combat exposed soldiers. *Psychoneuroendocrinology*. 2015;51:534-546. doi:10.1016/j.psyneuen.2014.07.010

359. Breen M, Maihofer A, Glatt S, et al. Gene Networks specific for innate immunity define post-traumatic stress disorder. *Mol Psychiatry*. 2015;20(12):1538-1545. doi:10.1038/mp.2015.9.Gene

360. Speer K, Upton D, Semple S, McKune A. Systemic low-grade inflammation in post-traumatic stress disorder: A systematic review. *J Inflamm Res*. 2018;11:111-121. doi:10.2147/JIR.S155903

361. Ménard C, Pfau ML, Hodes GE, Russo SJ. Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience. *Neuropsychopharmacol Rev*. 2017;42:62-80. doi:10.1038/npp.2016.90

362. Rajendran P, Chen Y, Chen Y, et al. The multifaceted link between inflammation and human diseases. *J Cell Physiplogy*. 2018;233:6458-6471. doi:10.1002/jcp.26479

363. Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. *Nat Rev Immunol*. 2017;17(1):49-59. doi:10.1038/nri.2016.123

364. Bekhbat M, Rowson SA, Neigh GN. Checks and balances : The glucocorticoid receptor and NF κ B in good times and bad. *Front Neuroendocrinol*. 2017;46:15-31. doi:10.1016/j.yfrne.2017.05.001

365. Michopoulos V, Powers A, Gillespie C, Ressler K, Jovanovic T. Inflammation in fear- and anxiety-based disorders: PTSD, GAD, and beyond. *Neuropsychopharmacology*. 2017;42:254-270.

366. Morey J, Boggero IA, Scott AB, Segerstrom SC. Current directions in stress and human immune function. *Curr Opin Psychol*. 2015;1(5):13-17. doi:10.1016/j.copsyc.2015.03.007.Current

367. Kim YK, Amidfar M, Won E. A review on inflammatory cytokine-induced alterations of the brain as potential neural biomarkers in post-traumatic stress disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;91:103-112. doi:10.1016/j.pnpbp.2018.06.008

368. Rusiecki JA, Byrne C, Galdzicki Z, et al. PTSD and DNA methylation in select immune function gene promoter regions : a repeated measures case-control study of U . S . military service members. *Front Psychiatry*. 2013;4(56):1-12. doi:10.3389/fpsyt.2013.00056

369. Ligthart S, Marzi C, Aslibekyan S, et al. DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. *Genome Biol*. 2016;17(225):1-15. doi:10.1186/s13059-016-1119-5

370. Cervantes JL, Weinerman B, Basole C, Salazar JC. TLR8 : the forgotten relative revindicated. *Cell Mol Immunol*. 2012;9:434-438. doi:10.1038/cmi.2012.38

371. Ma Y, Haynes R, Sidman R, Vartanian T. TLR8: AN innate immune receptor in brain, neurons and axons. *Cell Cycle*. 2007;6(23):2859-2868. doi:10.4161/cc.6.23.5018

372. Qu L, Ju JY, Chen SL, et al. Inhibition of the a -mannosidase Man2c1 gene expression enhances adhesion of Jurkat cells. *Cell Res*. 2006;16:622-631. doi:10.1038/sj.cr.7310065

373. Park H, Tan PS, Kavishna R, et al. Enhancing vaccine antibody responses by targeting Clec9A on dendritic cells. *npj Vaccines*. 2017;2(31):1-11. doi:10.1038/s41541-017-0033-5

374. Schreibelt G, Klinkenberg LJJ, Cruz LJ, et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-) presentation by human blood BDCA3 ♀ myeloid dendritic cells. *Immunobiology*. 2012;119(10):2284-2292. doi:10.1182/blood-2011-08-373944.The

375. Cimino PJ, Yang Y, Li X, et al. Ablation of the mocroglial protein DOCK2 reduces amyloid burden in a mouse model of Alzheimer's disease. *Exp Mol Pathol*. 2014;94(2):366-371. doi:10.1016/j.yexmp.2013.01.002.Ablation

376. Cimino PJ, Sokal I, Leverenz J, Fukui Y, Montine TJ. DOCK2 Is a Microglial Specific Regulator of Central Nervous System Innate Immunity Found in Normal and Alzheimer ' s Disease Brain. *Am J Pathol*. 2009;175(4):1622-1630. doi:10.2353/ajpath.2009.090443

377. Qureshi S, Kimbrell T, Pyne J, et al. Greater Prevalence and Incidence of Dementia in Older Veterans with Posttraumatic Stress Disorder. *J Am Geriatr Soceity*. 2010:1627-1633. doi:10.1111/j.1532-5415.2010.02977.x

378. Yaffe K, Vittinghoff E, Lindquist K, et al. Posttraumatic Stress Disorder and Risk of Dementia Among US Veterans. *Arch Gen Psychiatry*. 2010;67(6):608-613.

379. Penninx BWJH, Lange SMM. Metabolic syndrome in psychiatric patients: Overview, mechanisms, and implications. *Dialogues Clin Neurosci*. 2018;20(1):63-73.

380. Passos IC, Vasconcelos-Moreno MP, Costa LG, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *The Lancet Psychiatry*. 2015;2(11):1002-1012. doi:10.1016/S2215-0366(15)00309-0

381. Yang J-J, Jiang W. Immune biomarkers alterations in post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2020;268:39-46. doi:10.1016/j.jad.2020.02.044

382. Michels KB, Binder AM, Dedeurwaerder S, et al. Recommendations for the design and analysis of epigenome-wide association studies. *Nat Methods*. 2013;10(10):949-955. doi:10.1038/nmeth.2632

383. Mill J, Heijmans BT. From promises to practical strategies in epigenetic epidemiology. *Nat Rev Genet*. 2013;14(8):585-594. doi:10.1038/nrg3405

384. Rakyan V, Down T, Maslau S, et al. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res*. 2010;20(4):434–9. doi:10.1101/gr.103101.109.434

385. Hernandez DG, Nalls MA, Gibbs JR, et al. Distinct DNA methylation changes highly correlated with chronological age in the human brain. *Hum Mol Genet*. 2011;20(6):1164-1172. doi:10.1093/hmg/ddq561

386. Teschendorff AE, Menon U, Gentry-Maharaj A, et al. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res*. 2010;20(4):440-446. doi:10.1101/gr.103606.109

387. Bell JT, Tsai PC, Yang TP, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet*. 2012;8(4):e1002629. doi:10.1371/journal.pgen.1002629

388. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CPG island context. *PLoS Genet*. 2009;5(8):e1000602. doi:10.1371/journal.pgen.1000602

389. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3

390. Xiao FH, Wang HT, Kong QP. Dynamic DNA methylation during aging: A "prophet" of age-related outcomes. *Front Genet*. 2019;10:107. doi:10.3389/fgene.2019.00107

391. Yuan V, Price EM, Del Gobbo G, et al. Accurate ethnicity prediction from placental DNA methylation data. *Epigenetics and Chromatin*. 2019;12(1):1-14. doi:10.1186/s13072-019-0296-3

392. Galanter JM, Gignoux CR, Oh SS, et al. Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. *Elife*. 2017;6:e20532. doi:10.7554/eLife.20532

393. Evans GW, Kantrowitz E. Socioeconomic Status and Health: The Potential Role of Environmental Risk Exposure. *Annu Rev Public Health*. 2002;23(1):303-331. doi:10.1146/annurev.publhealth.23.112001.112349

394. Xia Y yin, Ding Y bing, Liu X qing, et al. Racial/ethnic disparities in human DNA methylation. *Biochim Biophys Acta*. 2014;1846:258-262. doi:10.1016/j.bbcan.2014.07.001

395. Chung WCJ, Auger AP. Gender differences in neurodevelopment and epigenetics. *Eur J Physiol*. 2013;465(5):573-584. doi:10.1007/s00424-013-1258-4

396. Nugent BM, McCarthy MM. Epigenetic underpinnings of developmental sex differences in the brain. *Neuroendocrinology*. 2011;93(3):150-158. doi:10.1159/000325264

397. Uddin M, Sipahi L, Li J, Koenen KC. Sex differences in dna methylation may contribute to risk of PTSD and depression: A review of existing evidence. *Depress Anxiety*. 2013;30(12):1151-1160. doi:10.1002/da.22167

398. Koukoura O, Sifakis S, Spandidos DA. DNA methylation in the human placenta and fetal growth (review). *Mol Med Rep*. 2012;5(4):883-889. doi:10.3892/mmr.2012.763

399. Huang K, Fan G. DNA methylation in cell differentiation and reprogramming: An emerging systematic view. *Regen Med*. 2010;5(4):531-544. doi:10.2217/rme.10.35

400. Edgar RD, Jones MJ, Meaney MJ, Turecki G, Kobor MS. BECon: A tool for interpreting DNA methylation findings from blood in the context of brain. *Transl Psychiatry*. 2017;7(8):e1187-10. doi:10.1038/tp.2017.171

401. Tylee DS, Kawaguchi DM, Glatt SJ. On the outside, looking in: A review and evaluation of the comparability of blood and brain "-omes." *Am J Med Genet Part B Neuropsychiatr Genet*. 2013;162(7):595-603. doi:10.1002/ajmg.b.32150

402. Braun PR, Han S, Hing B, et al. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. *Transl Psychiatry*. 2019;9(1). doi:10.1038/s41398-019-0376-y

403. Ratanatharathorn A, Aiello A, Boks M, et al. Epigenetic Biomarkers Of PTSD: Updates From The EWAS Working Group of The PTSD PGC. *Eur Neuropsychopharmacol*. 2019;29:S750. doi:10.1016/j.euroneuro.2017.06.092

404. Hannon E, Lunnon K, Schalkwyk L, Mill J. Interindividual methylomic variation across blood, cortex, and cerebellum: Implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics*. 2015;10(11):1024-1032. doi:10.1080/15592294.2015.1100786

405. Walton E, Hass J, Liu J, et al. Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. *Schizophr Bull*. 2016;42(2):406-414. doi:10.1093/schbul/sbv074

406. Adalsteinsson BT, Gudnason H, Aspelund T, et al. Heterogeneity in White Blood Cells Has Potential to Confound DNA Methylation Measurements. *PLoS One*. 2012;7(10):e46705. doi:10.1371/journal.pone.0046705

407. Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cells: Implications for cell lineage and studies on disease susceptibility. *PLoS One*. 2012;7:e41361. doi:10.1371/journal.pone.0041361

408. McGregor K, Bernatsky S, Colmegna I, et al. An evaluation of methods correcting for cell-type heterogeneity in DNA methylation studies. *Genome Biol*. 2016;17(1):1-17. doi:10.1186/s13059-016-0935-y

409. Teschendorff AE, Zheng SC. Cell-type deconvolution in epigenome-wide association studies: A review and recommendations. *Epigenomics*. 2017;9(5):757-768. doi:10.2217/epi-2016-0153

410. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13(1). doi:10.1186/1471-2105-13-86

411. Csoka AB, Szyf M. Epigenetic side-effects of common pharmaceuticals: A potential new field in medicine and pharmacology. *Med Hypotheses*. 2009;73:770-780. doi:10.1016/j.mehy.2008.10.039

412. Lee KWK, Pausova Z. Cigarette smoking and DNA methylation. *Front Genet*. 2013;4(JUL):1-11. doi:10.3389/fgene.2013.00132

413. LaSalle JM. A genomic point-of-view on environmental factors influencing the human brain methylome. *Epigenetics*. 2011;6(7):862-869. doi:10.4161/epi.6.7.16353

414. Zhang R, Miao Q, Wang C, et al. Genome-wide DNA methylation analysis in alcohol dependence. *Addict Biol*. 2013;18(2):392-403. doi:10.1111/adb.12037

415. Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. *Alcohol Res Curr Rev*. 2012;35(1):25-35.

416. Rosenbaum S, Stubbs B, Ward PB, Steel Z, Lederman O, Vancampfort D. The prevalence and risk of metabolic syndrome and its components among people with posttraumatic stress disorder: A systematic review and meta-analysis. *Metabolism*. 2015;64(8):926-933. doi:10.1016/j.metabol.2015.04.009

417. Barres R, Zierath JR. DNA methylation in metabolic disorders. *Am J Clin Nutr*. 2011;93(4):897-900. doi:10.3945/ajcn.110.001933

418. Dick KJ, Nelson CP, Tsaprouni L, et al. DNA methylation and body-mass index: A genome-wide analysis. *Lancet*. 2014;383:1990-1998. doi:10.1016/S0140-6736(13)62674-4

419. Althoff KN, McGinnis KA, Wyatt CM, et al. Comparison of risk and age at diagnosis of myocardial infarction, end-stage renal disease, and non-AIDS-defining cancer in HIV-infected versus uninfected adults. *Clin Infect Dis*. 2015;60(4):627-638. doi:10.1093/cid/ciu869

420. Nelson KN, Hui Q, Rimland D, et al. Identification of HIV infection-related DNA methylation sites and advanced epigenetic aging in HIV-positive, treatment-naive U.S. veterans. *Aids*. 2017;31(4):571-575. doi:10.1097/QAD.0000000000001360

421. LaMere SA, Chaillon A, Huynh C, Smith DM, Gianella S. Challenges in Quantifying Cytosine Methylation in the HIV Provirus. *Am Soceity Microbiol*. 2019;10(1):e02268. doi:10.1128/mBio.02268-18

422. Gornalusse GG, Mummidi S, Gaitan AA, et al. Epigenetic mechanisms, T-cell activation, and CCR5 genetics interact to regulate T-cell expression of CCR5, the major HIV-1 coreceptor. *Proc Natl Acad Sci U S A*. 2015;112(34):E4762-E4771. doi:10.1073/pnas.1423228112

423. Nakayama-Hosoya K, Ishida T, Youngblood B, et al. Epigenetic repression of interleukin 2 expression in senescent CD4+ T cells during chronic HIV type 1 infection. *J Infect Dis*. 2015;211(1):28-39. doi:10.1093/infdis/jiu376

424. Zhang Y, Li SK, Yi Yang K, et al. Whole genome methylation array reveals the down-regulation of IGFBP6 and SATB2 by HIV-1. *Sci Rep*. 2015;5:1-14. doi:10.1038/srep10806

425. Gross AM, Jaeger PA, Kreisberg JF, et al. Methylome-wide Analysis of Chronic HIV Infection Reveals Five-Year Increase in Biological Age and Epigenetic Targeting of HLA. *Mol Cell*. 2016;62(2):157-168. doi:10.1016/j.molcel.2016.03.019

426. Rickabaugh TM, Baxter RM, Sehl M, et al. Acceleration of age-associated methylation patterns in HIV-1-infected adults. *PLoS One*. 2015;10(3):1-18. doi:10.1371/journal.pone.0119201

427. Danese A, Moffitt T, Pariante C, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Arch Gen Psychiatry*. 2008;65(4):409-416. doi:10.1001/archpsyc.65.6.725

428. Danese A, Moffitt TE, Harrington HL, et al. Adverse childhood experiences and adult risk factors for age-related disease. *Arch Pediatr Adolesc Med*. 2009;163(12):1135-1143. doi:10.1001/archpediatrics.2009.214

429. Dudley KJ, Li X, Kobor MS, Kippin TE, Bredy TW. Epigenetic mechanisms mediating vulnerability and resilience to psychiatric disorders. *Neurosci Biobehav Rev*. 2011;35(7):1544-1551. doi:10.1016/j.neubiorev.2010.12.016

430. Suderman M, Borghol N, Pappas JJ, et al. Childhood abuse is associated with methylation of multiple loci in adult DNA. *BMC Med Genomics*. 2014;7(13). doi:10.1186/1755-8794-7-13

431. Unternaehrer E, Meyer AH, Burkhardt SCA, et al. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women. *Int J Biol Stress*. 2015;18(4):451-461. doi:10.3109/10253890.2015.1038992

432. Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. *PLoS One*. 2012;7(1):e30148. doi:10.1371/journal.pone.0030148

433. Wankerl M, Miller R, Kirschbaum C, Hennig J, Stalder T, Alexander N. Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. *Transl Psychiatry*. 2014;4:e402. doi:10.1038/tp.2014.37

434. Duman EA, Canli T. Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. *Biol*

Mood Anxiety Disord. 2015;5(1):1-14. doi:10.1186/s13587-015-0017-x

435. Shields AE, Wise LA, Ruiz-Narvaez EA, et al. Childhood abuse, promoter methylation of leukocyte NR3C1 and the potential modifying effect of emotional support. *Epigenomics*. 2016;8(11):1507-1517. doi:10.2217/epi-2016-0074
436. Gouin JP, Zhou QQ, Booij L, et al. Associations among oxytocin receptor gene (OXTR) DNA methylation in adulthood, exposure to early life adversity, and childhood trajectories of anxiousness. *Sci Rep*. 2017;7(1):1-14. doi:10.1038/s41598-017-07950-x
437. Janusek LW, Tell D, Gaylord-Harden N, Mathews HL. Relationship of childhood adversity and neighborhood violence to a proinflammatory phenotype in emerging adult African American men: An epigenetic link. *Brain Behav Immun*. 2017;60:126-135. doi:10.1016/j.bbi.2016.10.006
438. Nöthling J, Malan-Müller S, Abrahams N, Joanna Hemmings SM, Seedat S. Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review. *World J Biol Psychiatry*. 2019:1-20. doi:10.1080/15622975.2019.1583369
439. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296
440. Wiersma J, Hovens J, van Oppen P, et al. The Importance of Childhood Trauma and Childhood Life Events for Chronicity of Depression in Adults. *J Clin Psychiatry*. 2009;70(7):983-989.
441. Leverich GS, Mcelroy SL, Suppes T, et al. Early Physical and Sexual Abuse Associated with an Adverse Course of Bipolar Illness. *Soceity Biol Psychiatry*. 2002;51:288-297.
442. Alvarez M, Roura P, Oses A, Foguet Q, Sola J, Arrufat F. Prevalence and Clinical Impact of Childhood Trauma in Patients With Severe Mental Disorders. *J Nerv Ment Dis*. 2011;199(3):156-161. doi:10.1097/NMD.0b013e31820c751c
443. McLaughlin K, Green J, Gruber M, Sampson N, Zaslavsky A, Kessler R. Childhood adversities and adult psychopathology in the National Comorbidity Survey Replication (NCS-R) III: Associations with functional impairment related to DSM-IV disorders. *Psychol Med*. 2010;40(5):847-859. doi:10.1017/S0033291709991115.Childhood
444. Jonas S, Bebbington P, McManus S, et al. Sexual abuse and psychiatric disorder in England : Results from the 2007 Adult Psychiatric Morbidity Survey. *Psychol Med*.

2011:1-11. doi:10.1017/S003329171000111X

445. Afifi TO, Boman J, Fleisher W, Sareen J. Child Abuse & Neglect The relationship between child abuse , parental divorce , and lifetime mental disorders and suicidality in a nationally representative adult sample. *Child Abus Negl*. 2009;33:139-147. doi:10.1016/j.chiabu.2008.12.009
446. Campbell R, Sprague HB, Cottrill S, Sullivan CM. Longitudinal research with sexual assault survivors: A methodological review. *J Interpers Violence*. 2011;26(3):433-461. doi:10.1177/0886260510363424
447. Illumina. Infinium® MethylationEPIC BeadChip. *Illumina Mark*. 2015:4. https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/humanmethylationepic-data-sheet-1070-2015-008.pdf.
448. Smoller JW. The Genetics of Stress-Related Disorders: PTSD, Depression, and Anxiety Disorders. *Neuropsychopharmacology*. 2016;41(1):297-319. doi:10.1038/npp.2015.266
449. Wang Q, Shelton RC, Dwivedi Y. Interaction between early-life stress and FKBP5 gene variants in major depressive disorder and post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2018;225(July 2017):422-428. doi:10.1016/j.jad.2017.08.066
450. Ramo-fernández L, Boeck C, Koenig AM, et al. The effects of childhood maltreatment on epigenetic regulation of stress-response associated genes : an intergenerational approach. *Sci Rep*. 2019;9(983):1-12. doi:10.1038/s41598-018-36689-2
451. Paakinaho V, Makkonen H, Jääskeläinen T, Palvimo JJ. Glucocorticoid receptor activates poised FKBP51 locus through long-distance interactions. *Mol Endocrinol*. 2010;24(3):511-525. doi:10.1210/me.2009-0443
452. Binder EB, Bradley RG, Epstein MP, et al. Association of FKBP5 Polymorphisms and Childhood Abuse With Risk of Posttraumatic Stress Disorder Symptoms in Adults. *JAMA*. 2008;299(11):1291-1305.
453. Dunn EC, Solovieff N, Lowe SR, et al. Interaction between genetic variants and exposure to Hurricane Katrina on post-traumatic stress and post-traumatic growth: A prospective analysis of low income adults. *J Affect Disord*. 2014;152-154:243-249. doi:10.1016/j.jad.2013.09.018
454. Watkins LE, Han S, Harpaz-rotem I, et al. Psychoneuroendocrinology FKBP5 polymorphisms , childhood abuse , and PTSD symptoms : Results from the National

Health and Resilience in Veterans Study. *Psychoneuroendocrinology*. 2016;69:98-105. doi:10.1016/j.psyneuen.2016.04.001

455. Xie P, Kranzler HR, Poling J, Stein MB, Anton RF. Interaction of FKBP5 with Childhood Adversity on Risk for Post-Traumatic Stress Disorder. *Neuropsychopharmacology*. 2010;35:1684-1692. doi:10.1038/npp.2010.37

456. Young DA, Inslicht SS, Metzler TJ, Neylan TC, Ross JA. The e ff ects of early trauma and the FKBP5 gene on PTSD and the HPA axis in a clinical sample of Gulf War veterans ☆. *Psychiatry Res*. 2018;270(November 2017):961-966. doi:10.1016/j.psychres.2018.03.037

CHAPTER 2
EPIGENETIC ALTERATIONS ASSOCIATED WITH CHILDHOOD TRAUMA AND ADULT MENTAL HEALTH OUTCOMES: A SYSTEMATIC REVIEW

Published article:

This chapter presents the findings of a published systematic review on epigenetic alterations associated with childhood trauma and adult mental health outcome. The literature search was completed in March 2018. At the time there were a number of reviews investigating differential methylation in relation in psychiatric disorders, but none investigating childhood trauma, methylation and adult-onset psychiatric disorders. A number of new publications investigating childhood trauma and methylation have since been published, predominantly investigating glucocorticoid receptor nuclear receptor subfamily 3 (NR3C1) gene and supporting the conclusions drawn in this paper.

THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY
2020, VOL. 21, NO. 7, 493–512
https://doi.org/10.1080/15622975.2019.1583369

REVIEW ARTICLE

Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review

Jani Nothling[a], Stefanie Malan-Muller[a], Naeemah Abrahams[b], Sian Megan Joanna Hemmings[a] and Soraya Seedat[a]

[a]Department of Psychiatry, Stellenbosch University, Cape Town, South Africa; [b]Gender and Health Research Unit, South African Medical Research Council, Cape Town, South Africa

ABSTRACT

Objectives: Multiple, chronic and repeated trauma exposure in childhood is associated with adverse mental health outcomes in adulthood. In this paper we synthesise the literature on epigenetic modifications in childhood trauma (CT) and the mediating effects of differential epigenetic mechanisms on the association between CT and the later onset of psychiatric disorders.

Methods: We reviewed the literature up to March 2018 in four databases: PubMed, Web of Science, EBSCOhost and SCOPUS. Non-human studies were excluded. All studies investigating CT exposure both in healthy adults (18 years and older) and adults with psychiatric disorders were included.

Results: Thirty-six publications were included. For mood disorders, methylation of the glucocorticoid receptor *NR3C1* gene, specifically at the *NGFI-A* binding site in exon 1F, and correlation with CT was a robust finding. Several studies documented differential methylation of *SLC6A4*, *BDNF*, *OXTR* and *FKBP5* in association with CT. Common pathways identified include neuronal functioning and maintenance, immune and inflammatory processes, chromatin and histone modification, and transcription factor binding.

Conclusions: A variety of epigenetic mediators that lie on a common pathway between CT and psychiatric disorders have been identified, although longitudinal studies and consistency in methodological approach are needed to disentangle cause and effect associations.

ARTICLE HISTORY
Received 13 November 2018
Revised 28 January 2019
Accepted 11 February 2019

KEYWORDS
Adult mental health; child abuse; child neglect; childhood trauma; DNA methylation

1. Introduction

Childhood trauma (CT), including abuse and neglect, is prevalent across low-, middle-, and high-income countries, and can lead to a range of adverse physical, cognitive, social, emotional and behavioural outcomes during childhood (Kessler et al. 2010; Carr et al. 2013). CT increases the risk for adult-onset psychiatric disorders, including depression (Wiersma et al. 2009; Chen et al. 2010), bipolar disorder (BD) (Leverich et al. 2002; Alvarez et al. 2011), anxiety disorders (Chen et al. 2010; Hovens et al. 2009; McLaughlin et al. 2010), posttraumatic stress disorder (PTSD) (Chen et al. 2010; Jonas et al. 2011), personality disorders (Tyrka et al. 2009; Carr et al. 2013), alcohol and drug abuse (Jonas et al. 2011; Carr et al. 2013), eating disorders (Chen et al. 2010; Jonas et al. 2011), psychotic disorders (Üçok and Bikmaz 2007; Alvarez et al. 2011), and suicidal ideation and attempts (Afifi et al. 2009; Chen et al. 2010). An increased number of exposures is associated with a greater risk for adult psychiatric disorders, earlier age of diagnosis, increased number of hospital admissions, increased number of suicide attempts, younger age at first suicide attempt, greater symptom severity, and poorer prognosis in general (Kessler et al. 2010; Alvarez et al. 2011; Carr et al. 2013), while the co-occurrence of different types of CT (e.g., physical, sexual, emotional abuse and neglect) is also a common phenomenon (Kessler et al. 2010; Carr et al. 2013).

Although CT is established as a risk factor for adult psychiatric disorders, less is known about the mechanisms mediating the interplay between genetic and environmental factors, including factors that regulate gene expression (Lupien et al. 2009; Heim et al. 2010). One plausible mechanism linking early life experiences and adult mental health outcomes is an alteration to the epigenome. The epigenome is amenable to environmental influences that can alter gene expression profiles, without altering the genetic code itself (Vinkers et al. 2015; Mulligan 2016). The most

CONTACT Jani Nöthling janinothling@sun.ac.za Department of Psychiatry, Tygerberg Medical School, PO Box 241, Cape Town, 8000, South Africa

commonly investigated epigenetic mechanism in psychiatric disorders is DNA methylation, which involves the addition of a methyl group, predominantly to the fifth cytosine nucleotide (C-5) within cytosine-guanine or CpG dinucleotides (Tsankova et al. 2007; Grayson and Guidotti 2013; Klengel et al. 2014; Mulligan 2016). Methylation contributes to the condensation of chromatin, which restricts access to DNA and limits the binding of transcription factors at specific sites, which in turn causes transcriptional silencing (Moore et al. 2013). Demethylation of a genomic region, on the other hand, generally results in transcriptional activation (Brenet et al. 2011). Differential methylation of genes that transcribe and express proteins involved in the biological response to stress and trauma may underlie a range of phenotypic presentations (including behavioural and affective symptoms) (Grayson and Guidotti 2013; Klengel et al. 2014). Children are particularly vulnerable to the neuropsychological effects of trauma in view of heightened brain plasticity during critical developmental periods, which increases the likelihood of epigenomic alterations (Heim et al. 2010; Roth and Sweatt 2011). These alterations may impact structural and functional aspects of brain development and may have lifelong adverse effects on cognition and behaviour (Heim et al. 2010; Roth and Sweatt 2011).

The majority of methylation studies published to date have applied a candidate gene approach, where predetermined genes of interest are identified and investigated, based on prior knowledge of their involvement in disease or related biological functions (Harrison and Parle-McDermott 2011). More recent methylation studies have employed a genome-wide approach where CpG sites that cover the entire genome are scanned for epigenetic alterations (Bibikova et al. 2009; Bock et al. 2010). Global methylation, where average methylation levels are measured at intergenic regions and repetitive elements, has been investigated in a few studies (Beck and Rakyan 2008; Misiak et al. 2015). In this review, we systematically examine studies that have investigated epigenetic mechanisms in relation to CT and adult mental health outcomes.

2. Methods

A systematic, computerised search was conducted on 15 March 2018 using the following databases: PubMed, EBSCOhost, SCOPUS and Web of Science. The following search strategy was used: "(DNA methylation OR epigenetics OR epigenetic modifications OR epigenetic changes OR epigenetic alterations OR non-coding RNA OR nc-RNA OR micro-RNA OR miRNA OR histone modification) AND (Childhood abuse OR childhood neglect OR early life stress OR early life adversity OR childhood psychological trauma OR childhood physical abuse OR childhood sexual abuse OR childhood physical neglect OR childhood emotional neglect OR childhood emotional abuse OR childhood violence or childhood maltreatment)". No restrictions were placed on the initial search. All available sources were included in the search irrespective of their date or language of publication. Terms related to psychiatric disorders were not included in the search to avoid narrowing the results and confining it to specified disorders. The search was undertaken independently, on the same day, by two researchers (JN, SMM). Disagreements on eligibility were resolved by consensus.

Studies were excluded based on the following exclusion criteria: narrative or systematic review articles, book chapters, animal studies, in utero or birth-related traumas, studies that reported mental health outcomes in participants younger than 18 years. Only original studies were included in the review, while systematic and non-systematic reviews and book chapters were excluded. Studies that utilised non-human subjects were also excluded. Only studies investigating adults with CT were included. Studies that investigated later life mental health outcomes in participants younger than 18 years as well as those that investigated participants in which the age range included participants younger than 18 years (e.g., 16–24 years) were therefore excluded. No restrictions were placed on the type of epigenetic mechanism investigated or the type of CT experienced, as long as trauma exposure occurred after birth and not in utero or during birth. Studies of healthy adults with CT exposure as well as studies of adults with any psychiatric disorder with CT exposure were included. A standard data extraction form was used to collect detailed information on each study that met the inclusion criteria.

Thirty-six publications related to healthy adults and adults with depression, completed/attempted suicide, PTSD, borderline personality disorder (BPD), BD, generalised anxiety disorder (GAD), alcohol dependence and schizophrenia were retained and are discussed here. Some of the publications retained were drawn from the same study population and used overlapping samples, namely two publications from the Detroit Neighbourhood Health Study, four from the Grady Trauma Project, four from a Swiss BPD sample

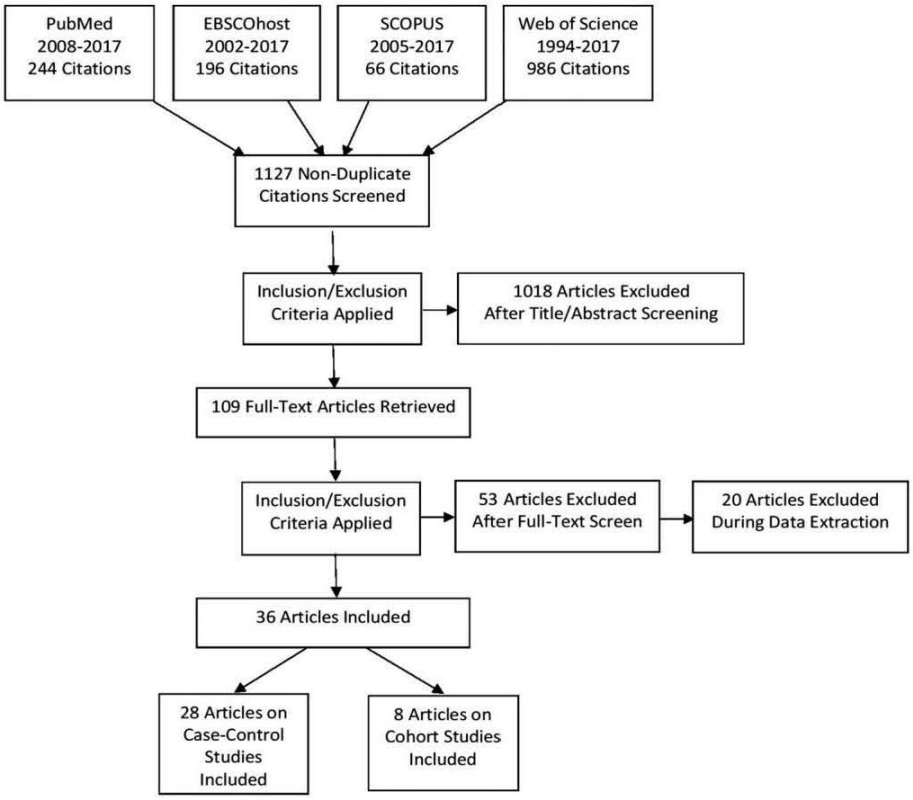


Figure 1. Data review and extraction flow diagram. Studies published up to 15 March 2018 were included.

receiving dialectical behavioural therapy and four from the Quebec suicide brain bank. In sum, this yielded 36 publications covering 26 samples. Figure 1 shows the flow of the data review and extraction process. Figure 2 presents the number of studies reviewed, categorised by the type of psychiatric disorder and the profile of genes that were investigated.

3. Results

Studies included in this review investigated candidate gene DNA methylation, global methylation and genome-wide methylation profiles. We first review studies in healthy adults with a history of CT, followed by a review of studies in adults with psychiatric disorders and CT. A detailed summary of each study is presented in Table 1.

3.1. Healthy adults

Eight studies investigated the relationship between CT and differential methylation in healthy adults. The majority of these studies specifically screened for and excluded participants with a psychiatric disorder and/or chronic physical diseases. Serotonin transporter gene/solute carrier family 6 *(SLC6A4)* and the glucocorticoid receptor gene/nuclear receptor subfamily 3, group C, member 1 *(NR3C1)* were investigated across several studies, while oxytocin receptor (*OXTR*), brain-derived neurotropic factor (*BDNF*) and interleukin 6 (*IL-6*) were investigated in single studies. Only one study investigated genome-wide methylation profiles. Findings are discussed in more detail below.

3.1.1. Serotonin transporter

Methylation of *SLC6A4,* involved in terminating neuronal absorption of serotonin was investigated in relation to CT in two studies using a sample of German Caucasians (n = 133) and North-American Caucasians (n = 105) (Wankerl et al. 2014; Duman and Canli 2015). No association between CT exposure and mean methylation levels across an overlapping *SLC6A4* promoter-associated CpG island was detected in these studies (Wankerl et al. 2014; Duman and Canli 2015). These findings are inconsistent with correlational data, suggesting that CT is linked to the dysregulation of serotonin which can in turn increase the risk for the development of various psychiatric disorders (De Bellis and Zisk 2014).

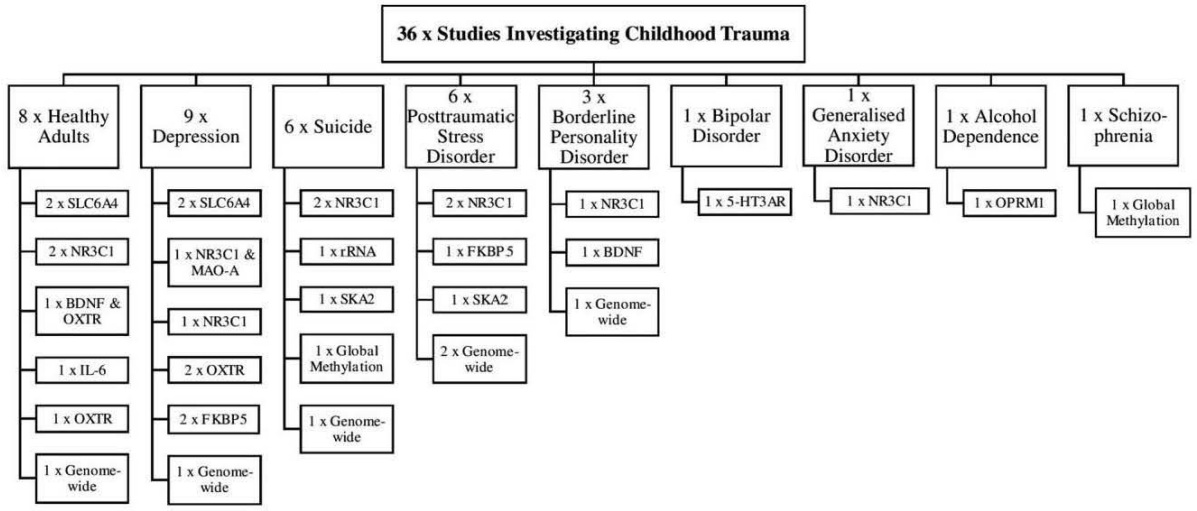


Figure 2. Number of studies included categorised by psychiatric outcome and gene investigated. Abbreviations: serotonin transporter gene/solute carrier family 6 (*SLC6A4*), glucocorticoid receptor gene/nuclear receptor subfamily 3, group C, member 1 (*NR3C1*), brain-derived neurotropic factor (*BDNF*), oxytocin receptor (*OXTR*), interleukin 6 (*IL-6*), monoamine oxidase A (*MAO-A*), FK506 binding protein (*FKBP5*), ribosomal RNA (*rRNA*), spindle and kinetochore associated complex subunit 2 (*SKA2*), serotonin receptor 3A (*5-HT3AR*), opioid receptor mu 1 (*OPRM1*).

3.1.2. Glucocorticoid receptor

Methylation of the *NR3C1* gene, implicated in the hypothalamic-pituitary-adrenal axis (HPA axis) regulation was investigated in two studies (Tyrka et al. 2012; Shields et al. 2016). In the first, African-American women (n = 295) with high levels of childhood physical abuse showed significantly increased methylation across a CpG island shore which originates in the *NR3C1* promoter (Shields et al. 2016). The second study investigated a region that encompasses *NR3C1* 1F promoter and includes a nerve growth factor-inducible protein A (*NGFI-A*) transcription factor binding site in a North-American sample (n = 99). The number of CTs experienced was associated with increased methylation levels at two individual CpG sites (one corresponded to the *NGF1-A* binding site) (Tyrka et al. 2012). While altered *NR3C1* methylation has been linked to various psychiatric disorders, these findings suggest that CT of itself may alter *NR3C1* methylation and expression and possibly mediate the risk for onset of psychiatric disorders (Shea et al. 2005; Heim et al. 2008; Faravelli et al. 2012; Klaassens et al. 2012; McCrory et al. 2012; Mehta and Binder 2012; Frodl and Keane 2013; Zorn et al. 2017).

3.1.3. Oxytocin receptor

One study investigated the relationship between maternal care and methylation of the *OXTR* gene which regulates the hormone and neurotransmitter oxytocin (Bartz et al. 2011; Skrundz et al. 2011).

Oxytocin is predominantly involved in the regulation of social interactions and bonding in relationships (Bartz et al. 2011; Olff et al. 2013). Swiss participants (n = 85) with low maternal care showed increased methylation across eight CpG sites in exon 3 of the *OXTR* gene (Unternaehrer et al. 2015). Another study investigated methylation of a promoter and enhancer region as well as intron 1 of the *OXTR* gene in a Canadian Caucasian sample of participants with and without CT (n = 46) (Gouin et al. 2017). Participants with CT had significantly increased methylation at one CpG site in the promoter region of the *OXTR* gene compared to those without CT; however, the statistical significance of the association was lost after correction for multiple testing (Gouin et al. 2017). Increased methylation of the *OXTR* gene may therefore be associated with adverse childhood experiences in healthy adults.

3.1.4. BDNF and IL-6

Methylation of *BDNF*, involved in neural development and maintenance, was investigated in a Swiss sample with low maternal care versus high maternal care (n = 85) (Unternaehrer et al. 2015). Participants with low maternal care showed significantly increased levels of *BDNF* methylation across 10 CpG sites in exon 6, independent of gender or age (Unternaehrer et al. 2015).

Methylation of the promoter region of *IL-6*, which is involved in inflammation, was investigated in relation

Table 1. Summary of epigenetic studies investigating childhood trauma and adult mental health outcomes.

Reference	Childhood trauma type and measure	Sample size and gender	Design and setting	Population	Diagnostic assessment	Ethnicity and mean age	Gene	Tissue type and technique	Main findings
(Wankerl et al. 2014)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	63 females, 70 Males	Case–control; community	Healthy adults	n.a.	German-Caucasian; 23.8 years	*SLC6A4*	Blood; bisulfite pyrosequencing	No significant effect of childhood trauma on *SLC6A4* methylation levels.
(Duman and Canli 2015)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	105 males	Case–control; community	Healthy adults	n.a.	Caucasian-American; 28.5 years	*SLC6A4*	Blood; EpiTYPER MassArray	No association between childhood trauma and *SLC6A4* methylation levels.
(Tyrka et al. 2012)	Low parental care, physical abuse, sexual abuse, emotional abuse, physical neglect, emotional neglect; Childhood Trauma Questionnaire	58 females, 41 males	Case–control; community	Healthy adults	n.a.	North American (not otherwise specified); 27.3 years	*NR3C1*	Blood; bisulfite pyrosequencing	[a]Increased methylation at the *NGF1-A* site in the *NR3C1* promoter region was associated with increased levels of childhood trauma.
(Shields et al. 2016)	Physical abuse and sexual abuse; Items from the Conflict Tactics Scale and the Pregnancy Abuse Assessment Screen	295 females	Cohort; community	Healthy adults	n.a.	African-American; 53.9 years	*NR3C1*	Blood; bisulfite pyrosequencing	[a]Women with childhood physical and sexual abuse showed significantly increased mean *NR3C1* methylation.
(Unternaehrer et al. 2015)	Level of maternal care; Parental Bonding Instrument	67 females, 18 males	Case–control; community	Healthy adults	n.a.	Swiss-European; 27.3 years	*BDNF*, *OXTR*	Blood; EpiTYPER MassArray	[a]Decreased maternal care was associated with significantly increased methylation in *BDNF* exon IV and *OXTR* exon III.
(Gouin et al. 2017)	Physical abuse, sexual abuse; Childhood Abuse Index	23 females, 23 females	Cohort; community	Healthy adults	n.a.	Canadian-Caucasian; 27 years	*OXTR*	Blood; bisulfite pyrosequencing	[a]Childhood trauma was associated with increased methylation at one CpG site in the promoter of the *OXTR* gene, but the association did not hold after correction for multiple testing.
(Janusek et al. 2017)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	34 males	Case–control; community	Healthy adults	n.a.	African-American; 20.2 years	*IL-6*	Blood; sodium bisulphite mapping	[a]Increased childhood trauma was associated with decreased methylation of *IL-6*.
(Suderman et al. 2014)	Verbal, emotional, physical and sexual abuse; binary response to individual questions	40 males	Cohort; birth cohort	Healthy adults	n.a.	British-Caucasian; 45 years	Genome-wide	Blood; methylated DNA immunoprecipitation and microarray hybridization (MeDIP)	[a]There were 311 hypermethylated and 686 hypomethylated gene promoter cites associated with child abuse.

(*continued*)

Table 1. Continued.

Reference	Childhood trauma type and measure	Sample size and gender	Design and setting	Population	Diagnostic assessment	Ethnicity and mean age	Gene	Tissue type and technique	Main findings
(Booij et al. 2015)	Physical abuse, sexual abuse and emotional abuse; Childhood Trauma Questionnaire	44 females, 25 males	Case–control; clinic	Depression	Structured Clinical Interview for DSM-IV Disorders	Irish, 37.8 years	*SLC6A4*	Blood; bisulfite pyrosequencing	[a]Increased levels of childhood abuse were significantly associated with increased *SLC6A4* methylation.
(Kang et al. 2013)	Parental loss, financial hardship, physical and sexual abuse; binary response to individual questions	81 females, 21 males	Cohort; clinic	Depression	Structured Clinical Interview for DSM Disorders	Korean, 54.9 years	*SLC6A4*	Blood; bisulfite pyrosequencing	[a]Parental loss, physical abuse, sexual abuse and child adversity in general were associated with significantly increased *SLC6A4* methylation.
(Melas et al. 2013)	Early parental death, parental divorce, financial problems and other familial constraints; binary response to four individual questions	174 females	Case–control; community	Depression	Major Depression Inventory	Swedish, 56 years (median age)	*NR3C1, MAO-A*	Saliva; EpiTYPER MassArray	[a]Childhood trauma was associated with significantly increased *NR3C1* methylation. Childhood adversity was not a significant predictor of *MAO-A* methylation.
(Bustamante et al. 2016)[1]	Physical abuse, sexual abuse and emotional abuse; items from the Conflict Tactics Scale and the Childhood Trauma Questionnaire	91 females, 56 males	Case–control; community	Depression	Patient Health Questionnaire	Mostly African-American; 49.6 years	*NR3C1*	Blood; bisulfite pyrosequencing	[a]Childhood trauma predicted significantly increased methylation in 4 CpG sites in the *NR3C1* gene.
(Smearman et al. 2016)[2]	Physical abuse, sexual abuse and emotional abuse; Childhood Trauma Questionnaire	275 females, 118 males	Cohort; community	Depression	Beck Depression Inventory	African-American; 41 years	*OXTR*	Blood; Illumina 450K BeadChip	[a]Childhood trauma was not associated with *OXTR* methylation. The interaction between childhood trauma and methylation predicted depression symptom severity.
(Kimmel et al. 2016)	Sexual abuse; binary response to one question	51 females	Case–control; clinic	Depression (postpartum)	DSM-IV criteria for current Major Depressive Episode	Mostly Caucasian-American; 30.6 years	*OXTR*	Blood; Illumina 450K BeadChip followed by bisulphite pyrosequencing	[a]Childhood trauma was associated with significantly increased *OXTR* methylation levels in women without postpartum depression, but not in woman with postpartum depression.
(Bustamante et al. 2018)[1]	Physical abuse, sexual abuse and emotional abuse; items from the Conflict Tactics Scale and the Childhood Trauma Questionnaire	62 females, 50 males	Case–control; community	Depression	Patient Health Questionnaire	Mostly African-American; 50.7 years	*FKBP5*	Blood; bisulfite pyrosequencing	Childhood trauma did not significantly predict *FKBP5* methylation across the promoter or at introns 2 and 7.
(Tozzi et al. 2018)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	66 female, 40 male	Case–control; not otherwise specified	Depression	Patient Health Questionnaire & Beck Depression Inventory	Irish; 37.6 years	*FKBP5*	Blood; bisulfite pyrosequencing	Childhood trauma was not a significant predictor of *FKBP5* methylation at intron 7.

(*continued*)

Table 1. Continued.

Reference	Childhood trauma type and measure	Sample size and gender	Design and setting	Population	Diagnostic assessment	Ethnicity and mean age	Gene	Tissue type and technique	Main findings
(Khulan et al. 2014)	Separated from parents due to World War II-related conditions	166 males	Case–control; birth cohort	Depression	Beck Depression Inventory	Finnish; 70.2 years	Genome-wide	Blood; Illumina 450K Array followed by bisulphite pyrosequencing	No significant differences in methylation profiles between those separated from their parents in childhood compared to those not separated from their parents.
(McGowan et al. 2009)[4]	Sexual abuse, physical abuse, and severe neglect; Childhood Experience of Care and Abuse	36 males	Case–control; Suicide Brain Bank	Suicide (completed)	n.a.	French-Canadian; 34.6 years	*NR3C1*	Hippocampal tissue; sodium bisulphite mapping	[a]Significant increased *NR3C1* methylation across promoter sites in suicide completers with childhood abuse.
(Labonte et al. 2012b)[4]	Sexual abuse, physical abuse, and severe neglect; Childhood Experience of Care and Abuse	56 males	Case–control; Suicide Brain Bank	Suicide (completed)	n.a.	French-Canadian; 34.6 years	*NR3C1*	Hippocampal tissue; bisulfite pyrosequencing	[a]No significant difference in methylation levels across exon 1B and 1C. Significantly decreased methylation across exon 1H in abused suicide completers.
(McGowan et al. 2008)[4]	Sexual abuse, physical abuse, and severe neglect; Childhood Experience of Care and Abuse	30 males	Case–control; Suicide Brain Bank	Suicide (completed)	n.a.	French-Canadian; 35 years	*rRNA*	Hippocampal tissue; sodium bisulphite mapping	[a]Significant increased methylation throughout the *rRNA* promoter region in abused suicide completers.
(Labont e et al. 2012a)[4]	Sexual abuse, physical abuse, and severe neglect; Childhood Experience of Care and Abuse	41 males	Case–control; Suicide Brain Bank	Suicide (completed)	n.a.	French-Canadian; 39.1 years	Genome-wide	Hippocampal tissue; custom-designed 400 micro-array followed by EpiTYPER MassArray	[a]Significant increased methylation in 248 promoters and significant decreased methylation in 114 promoters of abuse suicide completers.
(Kaminsky et al. 2015)[2]	Physical abuse, sexual abuse and emotional abuse; Childhood Trauma Questionnaire	196 females, 130 males	Cohort; community	Suicide (history of attempts)	Binary response to one question	Mostly African-American; ±30 years	*SKA2*	Blood and saliva; Illumina 450K BeadChip and bisulphite pyrosequencing	[a]Increased *SKA2* methylation interacted with increased childhood trauma scores to predict suicide attempts.
(Murphy et al. 2013)	Physical abuse and sexual abuse; documentation of a history of sexual or physical abuse	73 females, 86 males	Case–control; clinic	Suicide (history of attempts)	Documentation of a history of a completed act of self-harm	Irish; 37.2 years	Global	Blood; Methylflash methylated DNA quantification kit	History of childhood abuse was not a significant predictor of global methylation.

Table 1. Continued.

Reference	Childhood trauma type and measure	Sample size and gender	Design and setting	Population	Diagnostic assessment	Ethnicity and mean age	Gene	Tissue type and technique	Main findings
(Yehuda et al. 2016)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	24 females, 7 males	Case–control; Jewish cohort	Posttraumatic stress disorder	Clinician-administered PTSD Scale for DSM-IV disorders	Jewish-American; 46.5 years	*FKBP5*	Blood; bisulfite pyrosequencing	Childhood trauma was not associated with *FKBP5* methylation in the offspring of parents exposed and not exposed to the Holocaust.
(Boks et al. 2016)	General trauma, physical abuse, sexual abuse and emotional abuse; Early Trauma Inventory	93 males	Case–control; military cohort	Posttraumatic stress disorder	Self-Report Inventory for PTSD	European-Caucasian; 27.5 years	*SKA2*	Blood; Illumina 450K BeadChip	Childhood trauma was not significantly associated with a change in *SKA2* methylation levels.
(Smith et al. 2011b)[2]	Physical abuse, sexual abuse and emotional abuse; Childhood Trauma Questionnaire	40 females 64 males	Case–control; community	Posttraumatic stress disorder	Clinician-administered PTSD Scale for DSM-IV disorders	Mostly African-American; 42.7 years	Genome-wide	Blood; Illumina 27 BeadChip	There were no significant differences in methylation profiles of those with and without childhood trauma.
(Mehta et al. 2013)[2]	Physical abuse, sexual abuse and emotional abuse; Childhood Trauma Questionnaire	43 females, 18 males	Cohort; community	Posttraumatic stress disorder	Clinician-administered PTSD Scale for DSM-IV disorders	Mostly African-American; 41.63 years	Genome-wide	Blood; Illumina 450K BeadChip followed by EpiTYPER MassArray	[a]Participants with childhood trauma had only 2% overlap in gene expression profiles compared to those without childhood trauma.
(Perroud et al. 2011)[3]	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	170 females, 45 males	Case–control; clinic	Borderline personality disorder	Structured Clinical Interview for DSM-IV Disorders	Swiss; 36.6 years	*NR3C1*	Blood; bisulfite pyrosequencing	[a]Increased exposure to childhood trauma was associated with significantly increased *NR3C1* methylation.
(Perroud et al. 2013)[3]	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	132 females, 35 males	Case–control; clinic	Borderline personality disorder	Structured Clinical Interview for DSM-IV Disorders	Swiss; 35.5 years	*BDNF*	Blood; high-resolution melt assay	[a]Increased childhood trauma was associated with significantly increased *BDNF* methylation.
(Prados et al. 2015)[3]	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	147 females, 42 males	Case–control; clinic	Borderline personality disorder	Structured Clinical Interview for DSM-IV Disorders	Swiss; 36.8 years	Genome-wide	Blood; Illumina 450K BeadChip followed by bisulphite pyrosequencing	[a]Childhood trauma and borderline personality disorder was associated with differentially methylated genes related to inflammation processes; regulators of gene expression; neuronal and cell development, functioning and maintenance.
(Perroud et al. 2016)[3]	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	204 females, 145 males	Cohort; clinic	Bipolar disorder	Interview for Genetics Studies for DSM-IV disorders	Swiss-European; 38.1 years	*5-HT3AR*	Blood; bisulfite pyrosequencing	[a]Increased levels of emotional abuse, sexual abuse, physical abuse and physical neglect were associated with significantly decreased levels of methylation in one CpG site.

(*continued*)

Table 1. Continued.

Reference	Childhood trauma type and measure	Sample size and gender	Design and setting	Population	Diagnostic assessment	Ethnicity and mean age	Gene	Tissue type and technique	Main findings
(Wang et al. 2017)	Physical abuse, sexual abuse, emotional abuse, emotional neglect, physical neglect; Childhood Trauma Questionnaire	45 females, 19 males	Case–control; clinic	Generalised anxiety disorder	DSM-IV criteria for generalised anxiety disorder	Chinese; 35.4 years	*NR3C1*	Blood; sodium bisulfite mapping	[a]Higher levels of CT was associated with lower levels of NR3C1 1F promoter methylation across 38 CpG sites.
(Zhang et al. 2012)	Exposure to violence, physical abuse, sexual abuse, parental death; binary response to individual questions	79 females, 115 males	Case–control; clinic	Alcohol dependence	Semi-Structured Assessment for Drug Dependence and Alcoholism	Caucasian-American; 40 years	*OPRM1*	Blood; bisulfite Sanger sequencing	Childhood adversity did not significantly influence *OPRM1* promoter methylation levels.
(Misiak et al. 2015)	General trauma, physical abuse, emotional abuse, sexual abuse; Early Trauma inventory	52 females, 44 males	Case–control; clinic and community	Schizophrenia	Operational Criteria for Psychotic Illness Checklist	Polish; 26 years	*LINE-1* and *BAGE*	Blood; combined bisulfite restriction analysis (COBRA)	[a]Participants with first-episode schizophrenia and childhood trauma had significantly decreased *LINE-1* methylation levels.

[a]Significant findings related to childhood trauma reported; (1) overlapping samples from the Detroit Neighbourhood Health Study; (2) overlapping samples from the Grady Trauma Project; (3) overlapping samples from a Swiss clinic sample of participants receiving dialectical behavioural therapy; (4) overlapping samples form the Quebec suicide brain bank.

to CT in an African-American sample (n = 34) (Janusek et al. 2017). Higher levels of CT were associated with decreased methylation of *IL-6*, suggesting that CT may be linked to a proinflammatory phenotype that is often associated with mood and anxiety disorders in adulthood (Danesh et al. 2008; Hoge et al. 2008; Slopen et al. 2010; Janusek et al. 2017).

1.1.1. Genome-wide studies

One genome-wide study reported increased methyla- tion in 311 promoters and decreased methylation in 686 promoters in British-Caucasian participants with (n = 12) and without (n = 28) CT (Suderman et al. 2014). The differentially methylated genes were predominantly related to Wingless-type mouse mammary tumour virus (Wnt) signalling pathways, chromatin modification, histone modification, transcription factor binding, multicellular organism development and cell surface receptor-linked signal transduction. Increased methylation of 31 microRNAs and decreased methyla- tion of eight microRNAs were also associated with CT (Suderman et al. 2014). Increased levels of CT were positively associated with M20 domain-containing pro- tein 1 (*PM20D1*) (energy homeostasis regulator) methylation across several CpG sites (Suderman et al. 2014). Increased methylation of *PM20D1* has previously been linked to obesity, but little is known about its function in relation to psychiatric disorders (Feinberg et al. 2011; Suderman et al. 2014).

The aforementioned studies illustrate that CT may alter epigenetic profiles and ultimately gene and protein expression. In the section that follows, we discuss how these alterations in the context of CT can be linked to mental health outcomes.

1.2. Depression

Nine studies investigated the relationship between CT and differential methylation in adults with depression. *SLC6A4, NR3C1, OXTR* and FK506 binding protein *(FKBP5)* genes were investigated in more than one study, monoamine oxidase A (*MAO-A*) gene was investigated in one study only, and one study investigated genome-wide methylation profiles.

1.2.1. Serotonin transporter

Differential methylation was investigated in an overlapping regulating region of *SLC6A4* in two studies of Irish (n = 69) and Korean (n = 102) samples with clinical depression (Kang et al. 2013; Booij et al. 2015). Greater overall CT exposure was a significant predictor

of increased mean methylation of the *SLC6A4* promoter in both studies (Kang et al. 2013; Booij et al. 2015). These findings do not converge with the hypothesised role of increased serotonin transporter expression and decreased absorption of serotonin in the aetiology of depression (Brown and Harris 2008; Uher and McGuffin 2010; Carr and Lucki 2011).

3.2.2. Glucocorticoid receptor

CT significantly predicted increased average methylation at four CpG sites (two sites located at the *NGFI-A* binding site) in exon 1F of the *NR3C1* promoter in a study of African-American participants (n = 147) (Bustamante et al. 2016). This finding occurred independently of depression status, age, race, sex and antidepressant use (Bustamante et al. 2016). Similarly, parental death during childhood predicted increased *NR3C1* methylation at one CpG site, close to the *NGFI-A* binding site in a Swedish study (n = 74) (Melas et al. 2013). These findings correspond with those reported in healthy adults (Section 3.1.2) and suggest that CT may alter methylation and expression profiles of *NR3C1* (Tyrka et al. 2012; Shields et al. 2016).

3.2.3. Oxytocin receptor

Although CT was not associated with *OXTR* methylation in an African-American cohort (n = 393), increased severity of depression was significantly predicted by the interaction between CT and decreased methylation at two sites in exon 1 and increased methylation at one site in intron 3 in the same population (Smearman et al. 2016). A second study, in Caucasian-Americans, documented an association between CT and a significant increase in *OXTR* methylation in one CpG site, close to exon 3, but only in women who did not develop postpartum depression (i.e., healthy controls, n = 51) (Kimmel et al. 2016). The studies investigated different CpG sites and applied different methods which may explain the differences in findings.

3.2.4. FKBP5

CT was not a significant predictor of the average overall methylation of the *FKBP5* promoter or methylation in introns 2 and 7 in a predominantly African-American sample (n = 112) (Bustamante et al. 2018). Accordingly, *FKBP5* expression levels also did not differ between participants with and without CT (Bustamante et al. 2018). Similar results were generated for an Irish sample (n = 106), where no significant relationship was evident between CT and methylation levels of intron 7 of *FKBP5* (Tozzi et al. 2018). Both studies investigated an overlapping region of intron 7, including a glucocorticoid response element (Mehta et al. 2013). The results suggest that *FKBP5* methyla- tion, specifically at intron 7, does not mediate the relationship between CT and depression (Tozzi et al. 2018; Bustamante et al. 2018).

3.2.5. MAO-A

MAO-A, an enzyme that metabolises neurotransmitters (such as serotonin, dopamine and norepinephrine) was investigated in a Swedish sample (n = 174) (Melas et al. 2013). Decreased methylation of one CpG site in intron 1 was associated with depression status but not with CT (Melas et al. 2013). Decreased methylation of *MAO-A* may cause increased expression which, in turn, may contribute to the risk of developing depression; however, based on other studies methylation of *MAO-A* does not seem to mediate the relationship between CT and depression (Shulman et al. 2013; Meyer et al. 2017).

3.2.6. Genome-wide studies

One study compared Finish men who were separated from their parents during childhood with those who remained with their families during the Second World War (n = 166) (Khulan et al. 2014). Differential methylation was associated with mild depressive symptoms in both groups, but no significant differences in DNA methylation profiles were found between separated and non-separated offspring (Khulan et al. 2014).

3.3. Suicide

Six studies investigated the relationship between CT and differential methylation in adults who had committed/attempted suicide. *NR3C1* was investigated in two suicide studies using an overlapping dataset from the Quebec Suicide Brain Bank. The ribosomal RNA (*rRNA*) gene and spindle and kinetochore-associated complex subunit 2 (*SKA2*) gene, a chaperone of the glucocorticoid receptor, were investigated in single suicide studies only. One study investigated global methylation and another study genome-wide methylation.

3.3.1. Glucocorticoid receptor

Differential methylation of *NR3C1* was investigated using hippocampal tissue in a sample of French-Canadian suicide completers and controls (sudden deaths without medical intervention) (McGowan et al.

2009; Labont e et al. 2012b). Increased methylation across the *NR3C1* 1F promoter, encompassing 39 CpG sites (including the *NGFI-A* binding site), was reported in suicide completers exposed to CT compared to suicide completers without CT and controls (n = 36) (McGowan et al. 2009). The second study investigated mean methylation levels in promoter regions of non-coding exon 1B (across 12 CpG sites), 1C (across 18 CpG sites) and 1H (across 13 CpG sites) located in a CpG island in the *NR3C1* gene (n = 56) (Labonte et al. 2012b). Suicide completers with CT showed significantly decreased methylation across *NR3C1* exon 1H promoter compared to those without CT and controls (Labont e et al. 2012b). Suicide completers with CT also showed decreased 1H expression in hippocampal tissue (Labont e et al. 2012b). The findings in the first *NR3C1* (1F promoter) suicide study (McGowan et al. 2009) correspond with previous findings in healthy adults (Section 3.1.2) and adults with depression (Section 3.2.2) (Tyrka et al. 2012; Melas et al. 2013; Bustamante et al. 2016; Shields et al. 2016). Less is known about the influence of the promoter regions of exons 1B, 1C and 1H (as well as 1D, 1J and 1E) on glucocorticoid receptor functioning, given that these are non-coding exons (Qureshi and Mehler 2010; Qureshi and Mehler 2011; Qureshi and Mehler 2013).

3.3.2. rRNA and SKA2

Twenty-six CpG sites in the *rRNA* promoter region were investigated in hippocampal tissue in the same sample of suicide completers and controls from the Quebec Suicide Brain Bank (n = 30) (McGowan et al. 2008). Abused suicide completers had significantly increased mean methylation across CpG sites compared to controls (McGowan et al. 2008). Decreased expression of *rRNA* may contribute to decreased hippocampal volumes previously observed in individuals exposed to CT and increase the risk for various psychiatric disorders (Frodl and Keane 2013; Read et al. 2014; Harrisberger et al. 2015).

The relationship between suicide attempts, CT and *SKA2* methylation was investigated in one study of African-American participants (n = 326) (Kaminsky et al. 2015). *SKA2* methylation interacted with CT scores to predict suicide attempts (Kaminsky et al. 2015).

3.3.3. Global methylation

Global methylation in relation to CT was investigated in Irish participants with and without a history of suicide attempts (n = 159) (Murphy et al. 2013). Increased global methylation was a significant predictor of suicide attempts, independent of age, gender and smoking. However, increased global methylation was not associated with a history of CT (Murphy et al. 2013).

3.3.4. Genome-wide studies

One study investigated genome-wide methylation profiles, in the Quebec Suicide Brain Bank cohort (n = 41). Significantly increased methylation in 248 promoters and decreased methylation in 114 promoters were reported in suicide completers with CT compared to controls (Labont e et al. 2012a). The functional significance of the differentially methylated genes was investigated using cluster analysis, identifying a cluster related to cellular and neuronal plasticity (Labonte et al. 2012a). The alsin rho guanine nucleotide exchange factor two *(ALS2)* gene was associated with all sub-groups of the cellular and neuronal plasticity cluster (cell projection, neuron projection, cell soma and dendrites) and was investigated further (Labonte et al. 2012a). Suicide completers with CT showed significant hypermethylation across the *ALS2* gene, including the promoter area, compared to controls (Labont e et al. 2012a). Decreased *ALS2* expression has been linked to neurodegeneration and anxious behaviour in previous animal and human studies (Cai et al. 2005; Devon et al. 2006; Kasri and Van 2008; Pedrosa et al. 2010; Labont e et al. 2012a).

3.4. Posttraumatic stress disorder

Six studies investigated the relationship between CT and differential methylation of candidate genes, including *NR3C1, SKA2* and *FKBP5*, as well as genome-wide methylation profiles in adults with PTSD.

3.4.1. Glucocorticoid receptor

One study investigated a sample of Swiss women exposed to intimate partner violence and reported that physical abuse during childhood was significantly associated with decreased levels of *NR3C1* 1F mean methylation across 13 CpG sites (Schechter et al. 2015). Another study investigated a mixed ethnicity sample of male American war veterans and found no significant association between CT and mean *NR3C1* 1F methylation across 39 CpG sites (five sites overlapped with the first study) (Yehuda et al. 2015). Discrepant results may be explained by study differences related to gender, ethnicity, settings and methylation assays. Findings of studies in participants with PTSD also differed from healthy adults (Section 3.1.2),

adults with depression (Section 3.2.2) and suicide completers (Section 3.3.1), where CT was associated with increased *NR3C1* methylation levels (McGowan et al. 2009; Labont e et al. 2012b; Tyrka et al. 2012; Melas et al. 2013; Bustamante et al. 2016; Shields et al. 2016).

3.4.2. FKBP5 and SKA2 genes

One study investigated methylation of *FKBP5* in relation to CT and PTSD in Jewish participants (n = 31) (Yehuda et al. 2016). No significant association between CT and methylation of *FKBP5* across intron 7 was reported (Yehuda et al. 2016). This finding correlates with findings from studies investigating the relationship between depression and methylation of an overlapping region of *FKBP5* in intron 7 in this review (Section 3.2.4) (Tozzi et al. 2018; Bustamante et al. 2018).

The relationship between CT, *SKA2* methylation and PTSD was investigated in a study of male Dutch war veterans previously deployed to Afghanistan (n ¼ 93) (Boks et al. 2016). CT was not a significant independent predictor of change in *SKA2* methylation levels from pre-deployment to 6 months post-deployment, but pre-deployment *SKA2* methylation interacted with CT to predict PTSD (Boks et al. 2016). This finding corresponds with the finding reported by Kaminsky et al. (2015), who investigated the relationship between *SKA2* methylation (same CpG site), CT and suicide (Section 3.3.2) (Kaminsky et al. 2015).

3.4.3. Genome-wide studies

Methylation of CpG sites in five genes that have been predominantly implicated in inflammation and immune dysregulation was significantly associated with PTSD in one genome-wide study (n = 104) (Smith et al. 2011a). No single gene was significantly associated with CT on an experiment-wide level (Smith et al. 2011a). Another study investigated CT, PTSD and the relationship between gene expression and methylation (n = 61) (Mehta et al. 2013). The gene expression profiles of the group with PTSD and CT compared to the group with PTSD without CT were almost completely unique with only 2% overlap (Mehta et al. 2013). These findings suggest that CT exposure may have a large modifying effect on the methylation and gene expression profiles of PTSD patients and that different biological pathways may be impacted, in part dependent on the level of CT exposure.

3.5. Borderline personality disorder

Three studies investigated the relationship between CT, BPD and differential methylation in Swiss outpatients (Perroud et al. 2011; Perroud et al. 2013; Prados et al. 2015). Differential methylation of *NR3C1* and *BDNF* was reported in two separate papers. A third paper reported on genome-wide methylation results.

3.5.1. Glucocorticoid receptor and BDNF

CT was associated with increased methylation of *NR3C1* across eight CpG sites in the promoter of exon 1F, independent of BPD and comorbid pathology in the first BPD study (n = 215) (Perroud et al. 2011). Again, this finding corresponds to those reported in overlapping regions in healthy adults (Section 3.1.2), adults with depression (Section 3.2.2) and suicide completers (Section 3.3.1), but differs from that reported for PTSD (Section 3.4.1) (McGowan et al. 2009; Labonte et al. 2012b; Tyrka et al. 2012; Melas et al. 2013; Schechter et al. 2015; Yehuda et al. 2015; Bustamante et al. 2016; Shields et al. 2016).

The second study found that increased CT exposure was associated with hypermethylation of *BDNF* across nine CpG sites in exon 1 and 17 sites in exon 4 (n = 167) (Perroud et al. 2013). This finding is similar to that reported in healthy adults (Section 3.1.4) (Unternaehrer et al. 2015).

3.5.2. Genome-wide studies

The third genome-wide study documented differential methylation in genes related to inflammatory processes, regulators of gene expression, neuronal and cell development, functioning and maintenance (n = 189) in CT and BPD (Prados et al. 2015). Methylation of one CpG site near *miRNA-124* was explored, and increased CT scores were associated with hypomethylation of the *miRNA-124* CpG site (Prados et al. 2015). *MiRNA-124* targets glucocorticoid receptors and may therefore disrupt its translation and contribute to dysregulation of the HPA axis (Dwivedi 2014).

3.6. Bipolar disorder

One study investigated the relationship between CT, BD and differential methylation of the 5-hydroxytryptamine (serotonin) receptor 3A *(5-HT3AR)* in a Swiss sample (drawn from the same sample of participants as discussed in Section 3.5).

3.6.1. Serotonin receptor 3A

Increased levels of CT in participants with BD were associated with hypomethylation at one CpG site in the coding sequence of *5-HT3AR* (n = 349) (Perroud et al. 2016). The presence of a risk allele (rs1062613) in *5-HT3AR* was also associated with hypermethylation in participants with high levels of CT at a CpG site 1 bp upstream of this SNP (Perroud et al. 2013). This risk allele has been associated with schizophrenia and BD, but less is known about its interaction with CT, differential methylation and BD (Niesler et al. 2001; Frank et al. 2004; Hammer et al. 2012).

3.7. Generalised anxiety disorder

One study investigated the relationship between CT and methylation of the *NR3C1* 1F promoter in Chinese participants with and without GAD (Wang et al. 2017).

3.7.1. Glucocorticoid receptor

Higher levels of CT were associated with decreased methylation across 38 CpG sites in the *NR3C1* 1F promoter in participants with GAD (n = 64) (Wang et al. 2017). This finding differs from those reported in overlapping regions in healthy adults (Section 3.1.2), depressed patients (Section 3.2.2) and suicide completers (Section 3.3.1), but corresponds with results from PTSD studies (Section 3.4.1) (McGowan et al. 2009; Labont e et al. 2012b; Tyrka et al. 2012; Melas et al. 2013; Schechter et al. 2015; Yehuda et al. 2015; Bustamante et al. 2016; Shields et al. 2016; Wang et al. 2017).

3.8. Alcohol dependence

The relationship between methylation of the opioid receptor mu 1 (*OPRM1*) (involved in the reward pathway) CT and alcohol dependence was investigated in one study comprising a sample of Caucasian-Americans with alcohol dependence.

3.8.1. OPRM1

Methylation across the *OPRM1* promoter was significantly higher among participants with alcohol dependence; however, CT was not associated with *OPRM1* methylation (n = 194) (Zhang et al. 2012). These findings suggest that increased methylation of *OPRM1* may either predispose an individual to addiction or may be consequent to alcohol or drug abuse (Zhang et al. 2012).

3.9. Schizophrenia

One study investigated the relationship between CT, schizophrenia and global methylation in a Polish sample of first-episode schizophrenia patients and controls.

3.9.1. Global methylation

Long interspersed element 1 (*LINE-1*) and B melanoma antigen (*BAGE*) were used as indicators of global methylation (n = 96) (Misiak et al. 2015). Participants with schizophrenia and CT showed significantly decreased *LINE-1* methylation levels compared to participants with schizophrenia without CT (Misiak et al. 2015). Increased CT was also associated with decreased *BAGE* methylation in the whole sample (Misiak et al. 2015).

4. Discussion

Genes encoding components of the HPA axis were among the first to be investigated in epigenetic studies due to the well-established role of the HPA axis in the genesis of stress-related disorders, and its relation to CT (Heim et al. 2008; Faravelli et al. 2012; Heim and Binder 2012a; McCrory et al. 2012). Aptly, the glucocorticoid receptor gene *NR3C1* was the most widely studied gene in this review. Studies investigating depression, suicide, BPD and healthy adults have reported consistent associations between hypermethylation of the *NR3C1* exon 1F promoter region and exposure to CT, independent of the psychiatric disorder investigated (McGowan et al. 2009; Perroud et al. 2011; Tyrka et al. 2012; Melas et al. 2013; Bustamante et al. 2016; Shields et al. 2016; Perroud et al. 2011; Tyrka et al. 2012; Melas et al. 2013; Schechter et al. 2015; Bustamante et al. 2016). Methylation of an *NGFI-A* binding site, which directly interferes with *NR3C1* transcription and results in decreased glucocorticoid expression and altered cortisol production, was consistently associated with CT in healthy adults and adults with depression, BPD and attempted/completed suicide (Weaver et al. 2004; Weaver et al. 2007; Oberlander et al. 2008; McGowan et al. 2009; Weaver et al. 2014; Bustamante et al. 2016). Increased methylation of the exon 1F promoter was also associated with decreased expression of glucocorticoid receptor in a couple of studies (McGowan et al. 2009; Bustamante et al. 2016).

One PTSD study and one GAD study investigated methylation of the exon 1F promoter region and found the opposite relationship between CT and

NR3C1 methylation, where CT was associated with hypomethylation (McGowan et al. 2009; Perroud et al. 2011; Tyrka et al. 2012; Melas et al. 2013; Schechter et al. 2015; Bustamante et al. 2016; Shields et al. 2016; Wang et al. 2017). This is, however, in line with previous findings indicating that decreased expression of *NR3C1* is involved in the aetiology of mood disorders, while increased expression is generally linked to anxiety disorders (Shea et al. 2005; Heim et al. 2008; Faravelli et al. 2012; Klaassens et al. 2012; Mehta and Binder 2012; Zorn et al. 2017).

The serotonin transporter gene, *SLC6A4,* was the second most commonly investigated gene with consistent results in two healthy adult samples and two depression samples. All studies investigated an overlapping promoter associated CpG-rich region. Higher levels of CT were associated with *SLC6A4* hypermethylation in participants with depression, but no association between CT and *SLC6A4* methylation was reported in healthy adults (Kang et al. 2013; Wankerl et al. 2014; Booij et al. 2015; Duman and Canli 2015). Increased methylation in this promoter region is associated with decreased expression of serotonin transporter, which, in turn, increases uptake of serotonin (Kang et al. 2013; Booij et al. 2015). These findings suggest that CT is not associated with serotonin dysregulation in healthy adults but is associated with increased serotonin absorption in participants with depression. The findings differ from previous findings where decreased serotonin absorption due to CT has been associated with an increased risk for the development of psychiatric disorders (Domschke et al. 2014; Menke and Binder 2014).

The contradictory findings may be attributed to methodological differences, such as strict inclusion (physically healthy individuals) and exclusion (individuals presenting with current or past psychiatric symptoms) criteria applied in healthy adult samples, and may have resulted in an unusually resilient healthy adult sample. The inclusion of participants with depression receiving pharmacological treatment may have confounded *SLC6A4* methylation levels (Domschke et al. 2014; Menke and Binder, 2014).

BDNF was hypermethylated in relation to CT in two studies, one of which investigated differential methylation in exon 6 in healthy adults and the other in exons 1 and 4 in BPD patients (Perroud et al. 2013; Unternaehrer et al. 2015). Hypermethylation of *BDNF* (and a subsequent reduction in *BDNF* expression) may be implicated in a reduction of brain volume, which has been associated with CT in previous studies (Carrion et al. 2001; De Bellis et al. 2002; De Bellis and Kuchibhatla 2006; Pezawas et al. 2008; Bauer et al. 2009; Notaras et al. 2015). This is especially relevant in regions, such as the hippocampus and amygdala, where *BDNF* is frequently expressed (Carrion et al. 2001; De Bellis et al. 2002; De Bellis and Kuchibhatla 2006; Pezawas et al. 2008; Bauer et al. 2009; Notaras et al. 2015). Reduced brain volumes may interfere with long-term learning, memory and emotional processing, which may relate to symptom presentation in psychiatric disorders (Pezawas et al. 2008).

OXTR was hypermethylated in relation to CT in two studies, one investigating healthy adults and the other a healthy adult control sub-group in a depression study (Unternaehrer et al. 2015; Kimmel et al. 2016). Reduced oxytocin cerebrospinal fluid concentrations, possibly due to increased *OXTR* promoter methylation, have previously been linked to CT (Heim et al. 2009). Oxytocin administration has also been associated with decreased subjective stress ratings and decreased salivary cortisol levels, indicating that oxytocin possesses anxiolytic properties and interacts with the HPA axis (Hoge et al. 2008; Taylor et al. 2010; Olff et al. 2013). *OXTR* dysregulation may interfere with various social processes (e.g., attachment, emotion detection and recognition, trusting behaviour, affiliation and social motivation) and may mediate the relationship between CT and psychiatric disorders via these processes (Bartz et al. 2011; Skrundz et al. 2011).

FKBP5 was the only other gene with consistent findings across studies. CT was not associated with *FKBP5* methylation at an overlapping intron 7 region in one PTSD and two depression studies (Yehuda et al. 2016; Tozzi et al. 2018; Bustamante et al. 2018). *FKBP5* is activated in response to elevated glucocorticoid levels and *FKBP5* expression, in turn, suppresses glucocorticoid receptor activity by decreasing ligand binding and translocation of the receptor (Jaaskelainen et al. 2011; Szyf 2013). Previous studies have reported a link between differential methylation of *FKBP5*, disruption of the HPA axis and the development of psychiatric disorders. However, CT does not seem to mediate this relationship in PTSD and depression although studies using larger sample sizes may prove otherwise (Mehta et al. 2013; Szyf 2013).

PM20D1, *ALS2* and *miRNA-124, MAO-A*, *OPRM1*, *IL-6*, *rRNA* and *5-HT3AR* were investigated in single studies only (Labont e et al. 2012a; Suderman et al. 2014; Prados et al. 2015; McGowan et al. 2008; Zhang et al. 2012; Melas et al. 2013; Perroud et al. 2016; Yehuda et al. 2016; Janusek et al. 2017), while *SKA2* and global methylation were investigated in two studies each with conflicting findings (Murphy et al. 2013; Kaminsky

et al. 2015; Misiak et al. 2015; Boks et al. 2016). Further research is therefore needed to disentangle the relationship between these genes, global methylation and CT.

The majority of genome-wide methylation studies reported significant differences in methylation profiles of participants with and without CT (Labont e et al. 2012a; Mehta et al. 2013; Suderman et al. 2014; Prados et al. 2015). Differentially methylated genes were predominately associated with the following biological pathways: cell and neuronal functioning/maintenance, immune and inflammatory system, chromatin and histone modification and transcription factor binding (Labonte et al. 2012a; Mehta et al. 2013; Suderman et al. 2014; Prados et al. 2015). This indicates that CT may interfere with various biological pathways through methylation and may have lasting effects that manifest as poor mental health outcomes in adulthood.

A few limitations of the current review deserve mention. Studies published to date have generally been limited by the lack of statistical power stemming from small samples (Tsai et al. 2005). That said, increasing the sample size of epigenetic studies does not necessarily translate into increased power as the epigenome is not static like the genome: it adapts and changes due to cellular heterogeneity, gender, age, ethnicity and environmental factors (Mill and Heijmans 2013a). Power can be increased by reducing unwanted variability (through methodological and statistical methods) and undertaking longitudinal studies that allow for causality to be inferred and for the stability of epigenetic alterations to be tracked over time. Replication of findings reported in this review in independent datasets is also needed (Hunter 2005; Mill and Heijmans 2013a; Birney et al. 2016; Fiori and Turecki 2016; Lin et al. 2016; Zhao et al. 2016). Further, the lack of uniformity in defining CT, the lack of standardised measures of CT, and adjustment for confounding variables associated with CT should be addressed (Heim and Binder 2012b; Lopez-Castroman et al. 2012; Lopez-Castroman et al. 2013; Fiori and Turecki 2016; Brezo et al. 2017; Fergusson et al. 2000). The differences between studies in terms of molecular methods used to investigate DNA methylation may also limit the interpretability and generalisability of findings along with the limitations of popular molecular technologies (e.g., an inability to distinguish between methylated and hydroxymethylated cytosines following bisulphite treatment) (Yu et al. 2012; Pastor et al. 2013; Richa and Sinha 2014).

The functional significance of differential methylation at single CpG sites compared to methylation differences in larger regions remains unclear (Vinkers et al. 2015). Methylation levels at one CpG site are often highly correlated with methylation levels at other CpG sites that are in close proximity. The mean methylation levels across a number of CpG sites (e.g., a CpG island) may have a larger impact on expression levels than methylation at a single site (McGowan et al. 2011; Cao-Lei et al. 2013). In addition, the majority of studies were conducted in American, Canadian and European countries, with none investigating populations in developing countries. Including ethnically and geographically diverse cohorts in studies of epigenetic alterations in psychiatric disorders may contribute to a better understanding of the interplay of cultural, ethnic, socio-demographic factors, CT and methylation profiles in the context of mental health outcomes (Meloni 2017).

Lastly, studies investigating the relationship between CT and alternative epigenetic mechanisms, such as histone modification, ncRNAs and the interplay between RNAs, methylation and expression, are also lacking. Functional research is needed to elucidate the effects of alternative epigenetic mechanisms in the development of psychiatric disorders (Mill and Heijmans 2013a).

Advances have been made in identifying epigenetic risk factors and altered biological pathways that lie on the trajectory of CT and the development of psychiatric disorders. Refining the design and methodological strategy used in epigenetic studies may enhance our understanding of shared epigenetic alterations in psychiatric disorders, contribute to eliminating the noise caused by confounders, and enable the identification of mediators and moderators that are important in the pursuit of precision psychiatry. Identifying differentially methylated signatures across psychiatric disorders may also contribute to deciphering the complex interactions between different genes and their biological pathways and inform the development of targeted epigenetic therapies (Tsankova et al. 2007; Kubota et al. 2012).

Acknowledgements

None.

Statement of interest

None to declare.

Funding

This research is supported by: (i) The South African Research Chair in PTSD hosted by Stellenbosch University, funded by the Department of Science and Technology (DST) and administered by the South African National Research Foundation (NRF), and (ii) South African Medical Research Council in terms of the SAMRC's Flagships Awards Project SAMRC-RFA-IFSP-01-2013/RAPE COHORT. The first author is funded by the SAMRC under the National Health Scholars programme. The contents of the publication is solely the responsibility of the authors and do not necessarily represent the official views of the SAMRC.

ORCID

Jani Nothling http://orcid.org/0000-0001-6259-5810
Stefanie Malan-Muller http://orcid.org/0000-0003-0639-0129
Naeemah Abrahams http://orcid.org/0000-0002-6138-6256
Sian Megan Joanna Hemmings http://orcid.org/0000-0001-8461-1017
Soraya Seedat http://orcid.org/0000-0002-5118-786X

References

Afifi TO, Boman J, Fleisher W, Sareen J. 2009. The relationship between child abuse, parental divorce, and lifetime mental disorders and suicidality in a nationally representative adult sample. Child Abus Negl. 33:139–147.

Alvarez MJ, Roura P, Oses A, Foguet Q, Solà J, Arrufat FX. 2011. Prevalence and clinical impact of childhood trauma in patients with severe mental disorders. J Nerv Ment Dis. 199:156–161.

Bartz JA, Zaki J, Bolger N, Ochsner KN. 2011. Social effects of oxytocin in humans : context and person matter. Trends Cogn Sci. 15:301–309.

Bauer PM, Hanson JL, Pierson RK, Davidson RJ, Pollak SD. 2009. Cerebellar volume and cognitive functioning in children who experienced early deprivation. Biol Psychiatry. 66:1100–1106.

Beck S, Rakyan VK. 2008. The methylome: approaches for global DNA methylation profiling. Trends Genet. 24: 231–237.

Bibikova M, Le J, Barnes B, Saedinia-Melnyk S, Zhou L, Shen R, Gunderson KL. 2009. Genome-wide DNA methylation profiling using Infinium® assay. Epigenomics. 1:177–200.

Birney E, Smith GD, Greally JM. 2016. Epigenome-wide association studies and the interpretation of disease -omics. PLoS Genet. 12:1–9.

Bock C, Tomazou EM, Brinkman AB, Müller F, Simmer F, Gu H, Jäger N, Gnirke A, Stunnenberg HG, Meissner A. 2010. Quantitative comparison of genome-wide DNA methyla- tion mapping technologies. Nat Biotechnol. 28:1106–1114. Boks MP, Rutten BPF, Geuze E, Houtepen LC, Vermetten E, Kaminsky Z, Vinkers CH. 2016. SKA2 methylation is involved in cortisol stress reactivity and predicts the development of post-traumatic stress disorder (PTSD) after military deployment. Neuropsychopharmacol. 41: 1350–1356.

Booij L, Szyf M, Carballedo A, Frey EM, Morris D, Dymov S, Vaisheva F, Ly V, Fahey C, Meaney J. 2015. DNA methylation of the serotonin transporter gene in peripheral cells and stress-related changes in hippocampal volume: A study in depressed patients and healthy controls. PLoS One. 10:1–14.

Brenet F, Moh M, Funk P, Feierstein E, Viale AJ, Socci ND, Scandura JM. 2011. DNA methylation of the first exon is tightly linked to transcriptional silencing. PLoS One. 6: e14524.

Brezo J, Paris J, Barker ED, Tremblay R, Vitaro F, Zoccolillo M, Hebert M, Turecki G. 2017. Natural history of suicidal behaviors in a population-based sample of young adults. Psychol Med. 37:1563–1574.

Brown G, Harris TO. 2008. Depression and the serotonin transporter 5-HTTLPR polymorphism: A review and a hypothesis concerning gene-environment interaction. J Affect Disord. 111:1–12.

Bustamante AC, Aiello AE, Galea S, Ratanatharathorn A, Noronha C, Wildman DE, Uddin M. 2016. Glucocorticoid receptor DNA methylation, childhood maltreatment and major depression. J Affect Disord. [Internet]. 206:181–188.

Bustamante AC, Aiello AE, Guffanti G, Galea S, Wildman DE, Uddin M. 2018. FKBP5 DNA methylation does not mediate the association between childhood maltreatment and depression symptom severity in the Detroit Neighborhood Health Study. J Psychiatr Res. [Internet]. 96:39–48.

Cai H, Lin X, Xie C, Laird FM, Lai C, Wen H, Chiang H, Shim H, Farah MH, Hoke A. 2005. Loss of ALS2 Function Is Insufficient to Trigger Motor Neuron Degeneration in Knock-Out Mice But Predisposes Neurons to Oxidative Stress. Neurobiol Dis. 25:7567–7574.

Cao-Lei L, Suwansirikul S, Jutavijittum P, Meriaux SB, Turner JD, Muller CP. 2013. Glucocorticoid receptor gene expression and promoter CpG modifications throughout the human brain. J Psychiatr Res. [Internet]. 47:1597–1607.

Carr CP, Martins CMS, Stingel AM, Lemgruber VB, Juruena MF. 2013. The Role of Early Life Stress in Adult Psychiatric Disorders. J Nerv Ment Dis. [Internet]. 201:1007–1020.

Carr GV, Lucki I. 2011. The role of serotonin receptor subtypes in treating depression: A review of animal studies. Psychopharmacology. 213:265–287.

Carrion VG, Weems CF, Eliez S, Patwardhan A, Brown W, Ray RD, Reiss AL. 2001. Attenuation of frontal asymmetry in pediatric posttraumatic stress disorder. Biol Psychiatry. 50: 943–951.

Chen LP, Hassan Murad M, Paras ML, Colbenson KM, Sattler AL, Goranson EN, Elamin MB, Seime RJ, Shinozaki G, Prokop LJ, Zirakzadeh A. 2010. Sexual abuse and lifetime diagnosis of psychiatric disorders: systematic review and meta-analysis. Clin Proc. 85:618–629.

Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Higgins JPT, Lennon L, Eiriksdottir G. 2008. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: Two new prospective studies and a systematic review. PLoS Med. 5: 600–610.

De Bellis M, Zisk A. 2014. The biological effects of childhood trauma. Child. Adolesc Psychiatr Clin North Am. 23: 185–222.

De Bellis MD, Keshavan MS, Frustaci K, Shifflett H, Iyengar S, Beers SR, Hall J. 2002. Superior temporal gyrus volumes in maltreated children and adolescents with PTSD. Biol Psychiatry. 51:544–552.

De Bellis MD, Kuchibhatla M. 2006. Cerebellar volumes in pediatric maltreatment-related posttraumatic stress Disorder. Biol Psychiatry. 60:697–703.

Devon RS, Orban PC, Gerrow K, Barbieri MA, Schwab C, Cao LP, Helm JR, Bissada N, Davidson T, Witmer J. 2006. Als2-deficient mice exhibit disturbances in endosome trafficking associated with motor behavioral abnormalities. PNAS. 103:9595–9600.

Domschke K, Tidow N, Schwarte K, Deckert J, Lesch K, Arolt V, Zwanzger P, Baune BT. 2014. Serotonin transporter gene hypomethylation predicts impaired antidepressant treatment response. Int J Neuropsychopharmacol. 17: 1167–1176.

Duman EA, Canli T. 2015. Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. Biol Mood Anxiety Disord. [Internet]. 5:2.

Dwivedi Y. 2014. Emerging role of microRNAs in major depressive disorder: Diagnosis and therapeutic implications. Dialogues Clin Neurosci. 16:43–61.

Faravelli C, Lo Sauro C, Lelli L, Pietrini F, Lazzeretti L, Godini L, Benni L, Fioravanti G, Alina Talamba G, Castellini G, Ricca V. 2012. The Role of Life Events and HPA Axis in Anxiety Disorders: A Review. CPD. 18:5663–5674.

Feinberg AP, Irizarry RA, Fradin D, Aryee MJ, Murakami P, Aspelund T, Eiriksdottir G, Harris TB, Gudnason V, Fallin MD. 2011. Personalized epigenomic signatures that are stable over time and covary with body mass index. Sci Transl Med. 2:1–16.

Fergusson DM, Woodward LJ, Horwood LJ. 2000. Risk factors and life processes associated with the onset of suicidal behaviour during adolescence and early adulthood. Psychol Med. 30:23–39.

Fiori LM, Turecki G. 2016. Investigating epigenetic consequences of early-life adversity: some methodological considerations. Eur J Psychotraumatol. 7:1–10.

Frank B, Niesler B, Nothen M, Neidt H, Propping P, Bondy B, Frank B, Niesler B, No MM, Rietschel M, et al. 2004. the Human Serotonin Receptor Gene HTR3B in Bipolar Affective and Schizophrenic Patients. Am J Med Genet. 5: 1–5.

Frodl T, Keane VO. 2013. Neurobiology of disease how does the brain deal with cumulative stress? a review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol Dis. 52:24–37.

Gouin JP, Zhou QQ, Booij L, Boivin M, Côte SM, Hebert M, Ouellet-Morin I, Szyf M, Tremblay RE, Turecki G, Vitaro F. 2017. Associations among oxytocin receptor gene (OXTR) DNA methylation in adulthood, exposure to early life adversity, and childhood trajectories of anxiousness. Sci Rep. 7:1–14.

Grayson DR, Guidotti A. 2013. The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. Neuropsychopharmacol. 38:138–166.

Hammer C, Cichon S, Mühleisen TW, Haenisch B, Degenhardt F, Mattheisen M, Breuer R, Witt SH, Strohmaier J, Oruc L, et al. 2012. Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study. Transl Psychiatry. 2:e103.

Harrisberger F, Smieskova R, Schmidt A, Lenz C, Walter A, Wittfeld K, Grabe HJ, Lang UE, Fusar-Poli P, Borgwardt S. 2015. BDNF Val66Met polymorphism and hippocampal volume in neuropsychiatric disorders: A systematic review and meta-analysis. Neurosci Biobehav Rev. [Internet]. 55: 107–118.

Harrison A, Parle-McDermott A. 2011. DNA methylation: A timeline of methods and applications. Front Genet. 2: 632–649.

Heim C, Binder EB. 2012. Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene – environment interactions, and epigenetics. Exp Neurol. 233:102–111.

Heim C, Binder EB. 2012. Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene – environment interactions, and epigenetics. Exp Neurol. [Internet]. 233:102–111.

Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. 2008. The link between childhood trauma and depression: Insights from HPA axis studies in humans. Psychoneuroendocrinology. 33:693–710.

Heim C, Shugart M, Craighead WE, Nemeroff CB. 2010. Neurobiological and psychiatric consequences of child abuse and neglect. Dev Psychobiol. 52:671–690.

Heim C, Young L, Newport D, Mletzko T, Miller A, Nemeroff C. 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol Psychiatry. 14: 954–958.

Hoge EA, Pollack MH, Kaufman RE, Zak PJ, Simon NM. 2008. Oxytocin Levels in Social Anxiety Disorder. CNS Neurosci Ther. 14:165–170.

Hovens JGFM, Wiersma JE, Giltay EJ, Van Oppen P, Spinhoven P, Penninx BWJH, Zitman FG. 2009. Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. Acta Psychiatr Scand. 122:66–74.

Hunter DJ. 2005. Gene-environment interactions in human diseases. Nat Rev Genet. 6:287–298.

Jaaskelainen T, Makkonen H, Palvimo J. 2011. Steroid up-regulation of FKBP51 and its role in hormone signaling. Curr Opin Pharmacol. 11:326–331.

Janusek LW, Tell D, Gaylord-Harden N, Mathews HL. 2017. Relationship of childhood adversity and neighborhood violence to a proinflammatory phenotype in emerging adult African American men: An epigenetic link. Brain Behav Immun. [Internet]. 60:126–135.

Jonas S, Bebbington P, McManus S, Meltzer H, Jenkins R, Kuipers E, Cooper C, King M, Brugha T. 2011. Sexual abuse and psychiatric disorder in England: results from the 2007 Adult Psychiatric Morbidity Survey. Psychol Med. 41: 709–719.

Kaminsky Z, Wilcox HC, Eaton WW, Van Eck K, Kilaru V, Jovanovic T, Klengel T, Bradley B, Binder EB, Ressler KJ, Smith AK. 2015. Epigenetic and genetic variation at SKA2 predict suicidal behavior and post-traumatic stress disorder. Transl Psychiatry. 5:e627.

Kang HJ, Kim JM, Stewart R, Kim SY, Bae KY, Kim SW, Shin IS, Shin MG, Yoon JS. 2013. Association of SLC6A4 methylation with early adversity, characteristics and outcomes in

depression. Prog Neuro-Psychopharmacology Biol Psychiatry. 44:23–28.

Kasri NN, Van AL. 2008. Rho-linked genes and neurological disorders. Eur J Physiol. 455:787–797.

Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Aguilar-Gaxiola S, Alhamzawi AO, Alonso J, Angermeyer M, et al. 2010. Childhood adversities and adult psychopathology in the WHO world mental health surveys. Br J Psychiatry. 197:378–385.

Khulan B, Manning JR, Dunbar DR, Seckl JR, Raikkonen K, Eriksson JG, Drake AJ. 2014. Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current mental state. Transl Psychiatry. 4:e448.

Kimmel M, Clive M, Gispen F, Guintivano J, Brown T, Cox O, Beckmann MW, Kornhuber J, Fasching PA, Osborne LM, et al. 2016. Oxytocin receptor DNA methylation in postpartum depression. Psychoneuroendocrinology. [Internet]. 69:150–160.

Klaassens ER, Giltay EJ, Cuijpers P, van Veen T, Zitman FG. 2012. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: A meta-analysis. Psychoneuroendocrinology. 37:317–331.

Klengel T, Pape J, Binder EB, Mehta D. 2014. The role of DNA methylation in stress-related psychiatric disorders. Neuropharmacology. [Internet]. 80:115–132.

Kubota T, Miyake K, Hirasawa T. 2012. Epigenetic understanding of gene-environment interactions in psychiatric disorders: a new concept of clinical genetics. Clin Epigenetics. [Internet]. 4:1.

Labont e B, Suderman M, Maussion G, Navaro L, Yerko V, Mahar I, Bureau A, Mechawar N, Szyf M, Meaney MJ, Turecki G. 2012a. Genome-wide epigenetic regulation by early-life trauma. Arch Gen Psychiatry. [Internet]. 69: 722–731.

Labont e B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, Turecki G. 2012b. Differential glucocorticoid receptor exon 1 B, 1 C, and 1 H expression and methylation in suicide completers with a history of childhood abuse. Biol Psychiatry. [Internet]. 72:41–48.

Leverich GS, McElroy SL, Suppes T, Keck PE, Denicoff KD, Nolen WA, Altshuler LL, Rush AJ, Kupka R, Frye MA, et al. 2002. Early physical and sexual abuse associated with an adverse course of bipolar illness. Biol Psychiatry. 51: 288–297.

Lin X, Barton S, Holbrook JD. 2016. How to make DNA methylome wide association studies more powerful. Epigenomics. [Internet]. 8:1117–1129.

Lopez-Castroman J, Jaussent I, Beziat S, Genty C, Olie E, de Leon-Martinez V, Baca-Garcia E, Malafosse A, Courtet P, Guillaume S. 2012. Suicidal phenotypes associated with family history of suicidal behavior and early traumatic experiences. J Affect Disord. 142:193–199.

Lopez-Castroman J, Melhem N, Birmaher B, Greenhill L, Kolko D, Stanley B, Zelazny J, Brodsky B, Garcia-Nieto R, Burke AK, et al. 2013. Early childhood sexual abuse increases suicidal intent. World Psychiatry. 9:149–154.

Lupien SJ, McEwen BS, Gunnar MR, Heim C. 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 10:434–445.

McCrory E, De Brito SA, Viding E. 2012. The link between child abuse and psychopathology: A review of neurobiological and genetic research. J R Soc Med. 105:151–156.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labont e B, Szyf M, Turecki G, Meaney MJ. 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci. 12:342–348.

McGowan PO, Sasaki A, Huang TCT, Unterberger A, Suderman M, Ernst C, Meaney MJ, Turecki G, Szyf M. 2008. Promoter-wide hypermethylation of the ribosomal RNA Gene promoter in the suicide brain. PLoS One. 3:e2085.

McGowan PO, Suderman M, Sasaki A, Huang TCT, Hallett M, Meaney MJ, Szyf M. 2011. Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One. [Internet]. 6:e14739.

McLaughlin KA, Green JD, Gruber MJ, Sampson BA, Zaslavsky AM, Kessler RC. 2010. Childhood adversities and adult psychopathology in the National Comorbidity Survey Replication (NCS-R) III: associations with functional impairment related to DSM-IV disorders. Psychol Med. 40: 847–859.

Mehta D, Binder EB. 2012. Gene x environment vulnerability factors for PTSD: The HPA-axis. Neuropharmacology. 62: 654–662.

Mehta D, Klengel T, Conneely KN, Smith AK, Altmann A, Pace TW, Rex-Haffner M, Loeschner A, Gonik M, Mercer KB, et al. 2013. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. Proc Natl Acad Sci. [Internet]. 110: 8302–8307.

Melas PA, Wei Y, Wong CCY, Sjöholm LK, Åberg E, Mill J, Schalling M, Forsell Y, Lavebratt C. 2013. Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. Int J Neuropsychopharm. 16: 1513–1528.

Meloni M. 2017. Race in an epigenetic time: thinking biology in the plural. Br J Sociol. 68:389–409.

Menke A, Binder EB. 2014. Epigenetic alterations in depression and antidepressant treatment. Dialogues Clin Neurosci. 16:395–404.

Meyer J, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson A, Houle S. 2017. Elevated monoamine oxidase a levels in the brain. Arch Gen Psychiatry. 63:1209–1216.

Mill J, Heijmans BT. 2013a. From promises to practical strategies in epigenetic epidemiology. Nat Rev Genet. 14: 585–594.

Misiak B, Szmida E, Karpinski P, Loska O, Sąsiadek MM, Frydecka D. 2015. Lower LINE-1 methylation in first-episode schizophrenia patients with the history of childhood trauma. Epigenomics. [Internet]. 7:1275–1285.

Moore LD, Le T, Fan G. 2013. DNA Methylation and Its Basic Function. Neuropsychopharmacol. 38:23–38.

Mulligan CJ. 2016. Early environments, stress, and the epigenetics of human health. Annu Rev Anthropol. 45: 233–249.

Murphy TM, Mullins N, Ryan M, Foster T, Kelly C, McClelland R, O'Grady J, Corcoran E, Brady J, Reilly M, et al. 2013. Genetic variation in DNMT3B and increased global DNA methylation is associated with suicide attempts in psychiatric patients. Genes Brain Behav. 12:125–132.

Niesler B, Flohr T, Nothen M, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold G. 2001. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics. 11:471–475.

Notaras M, Hill R, Buuse M. Van Den 2015. The BDNF gene Val66Met polymorphism as a modi fi er of psychiatric disorder susceptibility: progress and controversy. Mol Psychiatry. 20:916–930.

Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. 2008. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics. 3:97–106.

Olff M, Frijling JL, Kubzansky LD, Bradley B, Ellenbogen MA, Cardoso C, Bartz JA, Yee JR, Zuiden M. Van 2013. The role of oxytocin in social bonding, stress regulation and mental health: An update on the moderating effects of context and interindividual differences. Psychoneuroendocrinology. [Internet]. 38:1883–1894.

Pastor WA, Aravind L, Rao A. 2013. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat Rev Mol Cell Biol. 14:341–356.

Pedrosa E, Shah A, Tenore C, Capogna M, Villa C, Guo X, Zheng D, Lachman HM. 2010. b-catenin promoter ChIP-Chip reveals potential schizophrenia and bipolar disorder gene network. J Neurogenet. 24:182–193.

Perroud N, Paoloni-Giacobino A, Prada P, Olie E, Salzmann A, Nicastro R, Guillaume S, Mouthon D, Stouder C, Dieben K, et al. 2011. Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. Transl Psychiatry. 1:e59.

Perroud N, Salzmann A, Prada P, Nicastro R, Hoeppli M-E, Furrer S, Ardu S, Krejci I, Karege F, Malafosse A. 2013. Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. Transl Psychiatry. 3:e207.

Perroud N, Zewdie S, Stenz L, Adouan W, Bavamian S, Prada P, Nicastro R, Hasler R, Nallet A, Piguet C, et al. 2016. Methylation of serotonin receptor 3A in ADHD, Borderline personality, and bipolar disorders: Link with severity of the disorders and childhood maltreatment. Depress Anxiety. 33:45–55.

Pezawas L, Meyer-Lindenberg A, Goldman AL, Verchinski BA, Chen G, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR. 2008. Evidence of biologic epistasis between BDNF and SLC6A4 and implications for depression. Mol Psychiatry. 13:709–716.

Prados J, Stenz L, Courtet P, Prada P, Nicastro R, Adouan W, Guillaume S, Oli e E, Aubry JM, Dayer A, Perroud N. 2015. Borderline personality disorder and childhood maltreatment: A genome-wide methylation analysis. Genes Brain Behav. 14:177–188.

Qureshi IA, Mehler MF. 2010. Emerging Role of Epigenetics in stroke: part 1: DNA methylation and chromatin modifications. Arch Neurol. 67:1316–1322.

Qureshi IA, Mehler MF. 2011. Non-coding RNA networks underlying cognitive disorders across the lifespan. Trends Mol Med. [Internet]. 17:337–346.

Qureshi IA, Mehler MF. 2013. Long Non-coding RNAs: Novel Targets for Nervous System Disease Diagnosis and Therapy. Neurotherapeutics. 10:632–646.

Read J, Fosse R, Moskowitz A, Perry B. 2014. The traumagenic neurodevelopmental model of psychosis revisited. Neuropsychiatry (London). [Internet]. 4:65–79.

Richa R, Sinha RP. 2014. Hydroxymethylation of DNA: an epigenetic marker. Excli J. 13:592–610.

Roth TL, Sweatt JD. 2011. Annual research review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. J Child Psychol Psychiatry Allied Discip. 52:398–408.

Schechter DS, Moser DA, Paoloni-Giacobino A, Stenz L, Gex-Fabry M, Aue T, Adouan W, Cordero MI, Suardi F, Manini A. 2015. Methylation of NR3C1 is related to maternal PTSD, parenting stress and maternal medial prefrontal cortical activity in response to child separation among mothers with histories of violence exposure. Front Psychol. [Internet]. 6:1–12.

Shea A, Walsh C, MacMillan H, Steiner M. 2005. Child maltreatment and HPA axis dysregulation: Relationship to major depressive disorder and post traumatic stress disorde in females. Psychoneuroendocrinology. 30:162–178.

Shields AE, Wise LA, Ruiz-Narvaez EA, Seddighzadeh B, Byun H-M, Cozier YC, Rosenberg L, Palmer JR, Baccarelli AA. 2016. Childhood abuse, promoter methylation of leukocyte *NR3C1*and the potential modifying effect of emotional support. Epigenomics. [Internet]. 8:1507–1517.

Shulman KI, Herrmann N, Walker SE. 2013. Current place of monoamine oxidase inhibitors in the treatment of depression. CNS Drugs. 27:789–797.

Skrundz M, Bolten M, Nast I, Hellhammer DH, Meinlschmidt G. 2011. Plasma oxytocin concentration during pregnancy is associated with development of postpartum depression. Neuropsychopharmacology. 36:1886–1893.

Slopen N, Lewis TT, Gruenewald TL, Mujahid MS, Ryff CD, Albert MA, Williams DR. 2010. Early life adversity and inflammation in african americans and whites in the midlife in the United States survey. Psychosom Med. 72: 694–701.

Smearman EL, Almli LM, Conneely KN, Brody GH, Sales JM, Bradley B, Ressler KJ, Smith AK. 2016. Oxytocin receptor genetic and epigenetic variations: association with child abuse and adult psychiatric symptoms. Child Dev. 87: 122–134.

Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley B, Tang Y, Gillespie CF, Cubells JF, KJ. 2011a. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. Am J Med Genet. 156B:700–708.

Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley B, Tang Y, Gillespie CF, Cubells JF, KJ. 2011b. Differential immune system DNA methyation and cytokine regulation in post-traumatic stress disorder. Am J Med Genet. 156B:700–708.

Suderman M, Borghol N, Pappas JJ, Pinto Pereira SM, Pembrey M, Hertzman C, Power C, Szyf M. 2014. Childhood abuse is associated with methylation of multiple loci in adult DNA. BMC Med Genomics. [Internet]. 7: 13.

Szyf M. 2013. How do environments talk to genes?. Nat Neurosci. 16:2–4.

Taylor SE, Saphire-Bernstein S, Seeman TE. 2010. Are plasma oxytocin in women and plasma vasopressin in men biomarkers of distressed pair-bond relationships? Psychol Sci. 21:3–7.

Tozzi L, Farrell C, Booij L, Doolin K, Nemoda Z, Szyf M, Pomares FB, Chiarella J, O'Keane V, Frodl T. 2018. Epigenetic changes of FKBP5 as a link connecting genetic and environmental risk factors with structural and functional brain changes in major depression. Neuropsychopharmacology. [Internet]. 43:1138–1145.

Tsai W-C, Hsu C-H, Lin C-C, Li Y-H, Lin L-J, Chen J-H. 2005. Correlation of decreased adiponectin with increased oxidative stress after a high-fat meal. Eur Heart J. 26:224.

Tsankova N, Renthal W, Kumar A, Nestler EJ. 2007. Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci. 8:355–367.

Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. 2012. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. PLoS One. 7:e30148.

Tyrka AR, Wyche MC, Kelly MM, Price LH, Carpenter LL. 2009. Childhood maltreatment and adult personality disorder symptoms: Influence of maltreatment type. Psychiatry Res. [Internet]. 165:281–287.

Üçok A, Bikmaz S. 2007. The effects of childhood trauma in patients with first-episode schizophrenia. Acta Psychiatr Scand. 116:371–377.

Uher R, McGuffin P. 2010. The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. Mol Psychiatry. 15: 18–22.

Unternaehrer E, Meyer AH, Burkhardt SCA, Dempster E, Staehli S, Theill N, Lieb R, Meinlschmidt G. 2015. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (*BDNF*) and oxytocin receptor (*OXTR*) in peripheral blood cells in adult men and women. Stress. [Internet]. 18: 451–461.

Vinkers CH, Kalafateli AL, Rutten BPF, Kas MJ, Kaminsky Z, Turner JD, Boks MPM. 2015. Traumatic stress and human DNA methylation: a critical review. Epigenomics. [Internet]. 7:593–608.

Wang W, Feng J, Ji C, Mu X, Ma Q, Fan Y, Chen C, Gao C, Ma X, Cang, Zhu F. 2017. Increased methylation of glucocorticoid receptor gene promoter 1Fin peripheral blood of patients with generalized anxiety disorder. J Psychiatr Res. [Internet]. 91:18–25.

Wankerl M, Miller R, Kirschbaum C, Hennig J, Stalder T, Alexander N. 2014. Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. Transl Psychiatry. 4: e402.

Weaver ICG, Alessio ACD, Brown SE, Hellstrom IC, Sharma S, Szyf M, Meaney MJ, Hospital D, Hh C. 2007. The Transcription Factor Nerve Growth Factor-Inducible Protein A mediates epigenetic programming: altering epigenetic marks by immediate-early genes. J Neurosci. 27: 1756–1768.

Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. 2004. Epigenetic programming by maternal behavior. Nat Neurosci. 7:847–854.

Weaver ICG, Hellstrom IC, Brown SE, Andrews SD, Dymov S, Diorio J, Zhang T-Y, Szyf M, Meaney MJ. 2014. The methylated-DNA binding protein MBD2 enhances NGFI-A (egr- 1)-mediated transcriptional activation of the glucocortic- oid receptor. Philos Trans R Soc B Biol Sci. 369: 20130513–20130513.

Wiersma JE, Hovens JGFM, van Oppen P, Giltay EJ, van Schaik DJF, Beekman ATF, Penninx BWJH. 2009. The importance of childhood trauma and childhood life events for chronicity of depression in adults. J Clin Psychiatry. 70: 983–989.

Yehuda R, Daskalakis NP, Bierer LM, Bader HN, Klengel T, Holsboer F, Binder EB. 2016. Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. Biol Psychiatry. [Internet]. 80:372–380.

Yehuda R, Flory JD, Bierer LM, Henn-Haase C, Lehrner A, Desarnaud F, Makotkine I, Daskalakis NP, Marmar CR, Meaney MJ. 2015. Lower Methylation of Glucocorticoid Receptor Gene Promoter 1F in Peripheral Blood of Veterans with Posttraumatic Stress Disorder. Biol Psychiatry. [Internet]. 77:356–364.

Yu M, Hon GC, Szulwach KE, Song C, Jin P, Ren B, He C. 2012. Tet-assisted bisulfite sequencing of 5-hydroxymethylcytosine. Nat Protoc. 7:2159–2170.

Zhang H, Herman AI, Kranzler HR, Anton RF, Simen A. a, Gelernter J. 2012. Hypermethylation of OPRM1 promoter region in European Americans with alcohol dependence. J Hum Genet. 57:670–675.

Zhao L, Fan C, Zhang Y, Yang Y, Wang D, Deng C, Hu W, Ma Z, Jiang S, Di S. 2016. Adiponectin enhances bone marrow mesenchymal stem cell resistance to flow shear stress through AMP-activated protein kinase signaling. Sci Rep. 6:28752.

Zorn JV, Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. 2017. Cortisol stress reactivity across psychiatric disor- ders: A systematic review and meta-analysis. Psychoneuroendocrinology 77:25–36.

CHAPTER 3

RISK AND PROTECTIVE FACTORS AFFECTING THE SYMPTOM TRAJECTORY OF POSTTRAUMATIC STRESS DISORDER POST-RAPE

ABSTRACT

Introduction: The prevalence of posttraumatic stress disorder (PTSD) in rape survivors is considerably higher than the prevalence in non-sexual trauma survivors. Few studies have investigated risk and protective factors in survivors early-after-rape in a prospective longitudinal design. **Methods:** Baseline data from a sample of 639 rape-exposed women assessed within 20 days of rape were analysed as putative predictors of PTSD symptom severity scores up to 6-months post-rape. **Results:** The incidence of PTSD at 3-months and at 6-months post-rape was 48.5% and 25.4%, respectively. Post-assault experience of rape stigma and depression were significant predictors of PTSD symptom scores in mixed linear regression models. Higher levels of depression and rape stigma at baseline (measured in quartile ranges) were associated with higher PTSD scores at all timepoints. **Conclusion:** Addressing rape stigma and the misattributions of rape in women who present to rape clinics may reduce the long-term adverse effects on mental health outcomes, such as PTSD. Rape survivors who present with high levels of depression soon after a rape should be carefully monitored and appropriately treated in order to reduce PTSD severity.

INTRODUCTION

In South Africa approximately 114 rape cases are reported to police every day with a total of 41,583 cases reported in the 2018/2019 financial year [1]. Crime statistics indicate that an average of 70.5 cases of rape are reported per 100 000 South African nationals [1,2]. There are no nationally representative studies on the under-reporting of rape, but regional survey data indicate that between 2.1% and 15.2% of women who have been raped report the incident to the police [3,4]. This suggests that the true prevalence of rape may be much higher than reflected in South African crime statistics [3,4].

Acute stress reactions are a normal response to interpersonal violence; however, symptoms reduce in severity and duration within the first four weeks following the event [5–7]. For some survivors of interpersonal violence, and specifically women exposed to sexual assault, traumatic stress responses may be sustained with high trauma symptomatology beyond 1-month post-exposure presenting as posttraumatic stress disorder (PTSD) [8–16]. Prospective studies investigating PTSD in sexual assault survivors have reported prevalence rates between 35% and 45% at 3-months post-rape [9,17–19] which are considerably higher than prevalence rates reported in general community samples that have investigated a mixture of trauma types [20–22]. Few studies to date have investigated rape and/or sexual assault beyond 3-months, but one study investigating a North American sample (n=126) of rape-exposed women reported a prevalence of 7.1% at 4-months post-assault [19] and another investigating a Swedish sample of rape-exposed women (n=317) reported a prevalence of 38.6% at 6-months post-rape [18].

Risk and protective factors for PTSD in sexual assault survivors, including rape, have been found to differ from those associated with non-sexual trauma types [9,23]. Prior studies have generally grouped risk and protective factors into pre-assault factors and post-assault factors [23,24]. Pre-assault factors include demographic factors (age, education, employment, relationship status, HIV status) and prior traumatic experiences (childhood trauma and lifetime trauma). The pre-assault factors: age [8,9,25], relationship status [18,25,26], education [8,26] and employment/income [8,18,25] have generally been found not to be associated with PTSD status and/or PTSD symptom severity across studies investigating sexual assault survivors. In some studies, however, younger age [26], being single [8] a lower level of education [25] and lower income [27] were associated with risk for developing PTSD. Studies also report a significant association between childhood trauma exposure [26–28], adult cumulative lifetime trauma [9,18,26], HIV status[29] and PTSD, while others have not replicated these finding [8,18].

Post-assault risk (perceived stress, rape stigma, alcohol use, depression) and protective factors (resilience, social support) may also contribute to PTSD symptom severity [8,9,18,26,27,30,31]. Higher levels of perceived stress [5,32] have been found to predict PTSD symptom severity following trauma exposure. Negative social reactions and rape stigma (e.g. victim blaming, self-blaming, shame, embarrassment, downplaying the severity of the rape, treating the victim differently) have also been associated with PTSD symptom severity in a number of studies [10,25,26,31]. Lower levels of social support have been associated with higher PTSD symptom severity in one cross-sectional rape study [33] while others did not replicate this finding [8,9]. Factors associated with low resilience such as negative affectivity (general negative perspective on the world and oneself), avoidance coping (disengagement, denial and self-distraction), loss of control (related to emotional recovery and future victimisation) and negative beliefs about personal characteristics (being careless, unlucky, too trusting and a victim type) have generally been associated with increased PTSD symptom severity across rape studies [9,26,31].

Although depression and alcohol use are referred to as post-assault risk factors in this study, they can be both pre-assault and post-assault risk factors for PTSD. Increased alcohol use and depression severity has been found to covary/predict PTSD status/symptom severity post-rape [8,18,27,34–39], but depression and alcohol use have also been associated with an increased risk for sexual assault exposure [34–36,40,41]. Pre-existing depression has been associated with a three-fold increased risk for PTSD following trauma exposure while a 2.8-fold increased risk for first onset depression has been reported in participants exposed to interpersonal violence (including sexual assault) and with PTSD compared to those who did not develop PTSD following trauma exposure [34]. A history of sexual assault and increased risk for the development of PTSD have been associated with problematic alcohol use prior to the assault and at the time of the assault [35,37,38]. One study found that 26.3% of sexual assault survivors showed problematic alcohol use prior to the assault, 17.5% showed problematic alcohol use at the same age at which the sexual assault occurred and 56.1% developed problematic alcohol use following the assault [35].

Pre-assault and post-assault factors may play a considerable role in the risk of developing PTSD 1-month post-rape, but may also have long-term effects on the trajectory of PTSD symptomatology [5,7,23]. Trauma type itself may influence the trajectory of PTSD e.g. traumas involving intentional injury or harm have been found to result in higher PTSD prevalence at 1-month, 3-months, 6-months and 12-months post-rape compared to trauma associated with non-intentional injury [7]. Limited access to mental health resources as a result

of poverty [42]; the adverse effects of childhood trauma or cumulative lifetime trauma [42–44]; negative coping mechanisms such as social isolation and alcohol abuse [43–45]; stigmatising views on rape [8,10,18,27,31,45–48] and predisposition to anxiety and depression [43–45] may sustain PTSD symptoms and result in an upward PTSD symptom trajectory and chronicity.

Fewer studies have investigated factors associated with the assault itself. Assault-factors such as the severity of the rape and specific contextual factors related to the rape may influence the degree of fear, horror and life threat experienced by rape survivors [18,25,26,30,48]. Multiple perpetrators [18], multiple sexual acts [18], physical injury inflicted during the rape [18,25] and a longer duration of assault [30] have generally been found to be associated with PTSD. However, other studies have not found a significant relationship between the number of perpetrators [9], physical injury inflicted [8,26] and general assault severity [26]. Relationship with the perpetrator (e.g. family member, partner, friend, acquaintance, stranger) has been found not to be associated with PTSD symptom severity or status [8,9,18,25]. Some studies reported a significant association between perceived life threat during the assault and PTSD symptom severity [25,26,30] while one study did not [18].

Across studies investigating sexual violence, findings in relation to risk and protective factors have been inconsistent. Few studies have comprehensively investigated pre- and post-assault risk and protective factors for PTSD in a longitudinal prospective design, especially beyond the 3-month post-rape period and in low- to medium-income countries [18,23]. In this study we sought to investigate: (1) the relationship between baseline (within 20 days post-rape) pre- and post-assault factors in relation to PTSD symptom severity at 6-months post-rape in a cohort of rape-exposed women residing in a low-income region of South Africa; (2) the interaction between baseline pre- and post-assault variables and PTSD symptom scores over time (baseline, 3-months and 6-months post-rape) and; (3) the association between assault related variables and PTSD symptom severity at baseline, 3-month and 6-months post-rape in a sub-sample of rape-exposed participants who disclosed assault-related details.

METHODS

Participants and setting

Rape-exposed participants (n = 782) were recruited for the parent study *"Rape Impact Cohort Evaluation (RICE)"*, a study investigating the impact of rape on women's health and their use of health services in South Africa [49]. Female survivors of rape were recruited from four rape centres in and around the city of Durban located in the South African province of KwaZulu-

Natal. The rape centres provide comprehensive emergency care, including access to police, counselling, medical and forensic care. Rape survivors who presented to one of four rape clinics were informed of the study after they had received post-rape care and had the initial clinical and forensic assessment. Interested participants were invited to the study site to obtain more information and to enrol in the study.

Recruitment was restricted to female participants between 16 and 40 years of age who had reported a rape in the past 20 days. Participants were excluded if they were severely distressed or in need of urgent psychological or psychiatric attention/hospitalisation (in which case they were supported and/or referred by the counsellor at the rape centre where the rape was initially reported); intellectually disabled; or more than 14 weeks pregnant. In order to investigate predictors of PTSD associated with rape, we excluded participants who met criteria for PTSD at baseline due to traumatic events other than the current rape (n=143).

Study procedures

Study procedures were explained to participants and informed consent was obtained by research assistants trained in research ethics and conducting research in vulnerable populations. At the baseline visit (within 20 days post-rape), participants completed a demographic questionnaire; a psychiatric interview; self-report questionnaires to gather data on the rape incident, childhood and lifetime trauma, resilience, social support, perceived stress, rape stigma, alcohol use, depression and PTSD (participants were asked to endorse PTSD symptoms in relation to the rape on the PTSD measure). The aforementioned measures are described in Table 1 along with the Cronbach alpha test statistic obtained from the data in this study and variables used in the analyses. A rapid HIV antibody blood spot test and a human chorionic gonadotropin (hCG) urine pregnancy test was also completed at baseline. The demographic questionnaire and self-report measures were administered by research assistants. The psychiatric interview was also administered by research assistants under the supervision of a registered trauma counsellor or nurse. The medical procedures were administered by a registered nurse. All assessments were completed face-to-face and responses to the demographic questionnaire and self-report measures were electronically recorded and captured in real-time. All mental health assessments, except for the time-independent demographic and childhood/lifetime trauma related measures, were repeated at the 3- and 6-month follow-up visits.

Ethical considerations

The study was introduced to potential participants as the 'Women's Health and wellbeing study' in order to protect them from being identified as someone who has been raped. Participants were informed that participation is voluntary and that they were allowed to withdraw from the study at any time. All data gathered in the study were deidentified by replacing any identifiable information with a study number. The study staff received training in ethical and legal issues associated rape. A counsellor specialising in trauma counselling was onsite to assist participants who exhibited signs of distress and to refer participants in need of specialised psychological or psychiatric care. Ethical approval to conduct the RICE study was obtained from the South African Medical Research Council Ethics Committee (SAMRC; EC019-10/2013) and approval to conduct the sub-study was obtained from the Health Research Ethics Committee at Stellenbosch University (HREC; S16/08/146).

Table 1: *Description of measures and variables used in the study*

Measure	Description	Timepoint/s administered	Cronbach alpha	Variable/s	Source of Measure
Demographic questionnaire	Six items with diverse response options depending on individual items	Baseline		(1) Age, (2) education, (3) employment, (4) relationship status, (5) HIV status	(Jewkes et al., 2006)[50]
Mini International Neuropsychiatric Interview version 7.0.0 (MINI) – PTSD module	Twenty-one DSM-5 symptoms. Subscales include criterion A (stressor), criterion B (intrusion symptoms), criterion C (avoidance), criterion D (negative alterations in cognitions and mood), criterion E (alterations in arousal and reactivity). Responses are recorded as 0 ('no') or 1 ('yes') for presenting with a symptom. One or more symptoms endorsed in criterion A, B and C respectively and 2 or more symptoms endorsed in criterion D and E respectively constitutes a PTSD diagnosis.	Baseline		PTSD status	(Sheehan et al., 2014)[51]
Childhood Trauma Questionnaire - Short Form (CTQ-SF)[1]	Fourteen items e.g. 'before I reached 18, I was beaten so hard at home that it left a mark or bruise'. Subscales include sexual abuse, physical abuse, emotional abuse, neglect and domestic violence. Responses recorded on a 4-point Likert scale ranging from 1 ('never') to 4 ('very often'). Total score rage between14 and 56.	Baseline	.80	Childhood trauma	(Bernstein & Fink, 1998)[52]
Life Events Checklist (LEC)[1]	Ten items e.g. 'Have you ever experienced the murder of a family or friend?'. Responses recorded as 0 ('no') or 1 ('yes'). Total score rage between 0 and 10.	Baseline		Lifetime cumulative trauma: (1) imprisonment, (2) civil unrest/war, (3) serious injury, (4) being close to death, (5) murder of family or friend, (6) unnatural death of family or friend, (7) murder of stranger, (8) robbed at gunpoint/ knifepoint (9) kid-napping, (10) sexual assault	(Weathers et al., 2013)[53]
Davidson Trauma Scale (DTS)	Seventeen items e.g. 'have you been upset by something which reminded you of the event?'. Subscales include symptom frequency and symptom severity. Responses	Baseline 3-months 6-months	.95	PTSD symptom score	(Davidson et al., 1997)[54]

	recorded on a 5-point Likert scale for symptom frequency ranging from 0 ('not at all') to 4 ('every day') and symptom severity ranging from 0 ('not at all distressing') to 4 ('extremely distressing'). Total score range between 0 and 136. A cut-off of 40 indicates clinically significant PTSD symptoms.				
The Connor-Davidson Resilience Scale (CD-RISC)	Twenty-five items e.g. 'I am able to adapt when changes occur'. Responses recorded on a 4-point Likert scale ranging from 1 ('strongly disagree') to (4 'strongly agree')[2]. Total score range between 25 and 100.	Baseline 3-months 6-months	.89	Resilience	(Connor & Davidson, 2003)[55]
Multidimensional Scale of Perceived Social Support (MSPSS)	Twelve items e.g. 'There is a special person who is around when I am in need'. Responses recorded on a 4-point Likert scale ranging from 1 ('strongly disagree') to 4 ('strongly agree')[2]. Total score range between 12 and 48.	Baseline 3-months 6-months	.88	Social Support	(Zimet, Dahlem, & Farley, 1988)[56]
Perceived Stress Scale (PSS)	Ten items e.g. 'In the last month, how often have you found that you could not cope with all the things that you had to do?'. Responses recorded on a 4-point Likert scale ranging from 1 ('strongly disagree') to (4 'strongly agree')[2]. Total score range between 9 and 36.	Baseline 3-months 6-months	.53	Perceived stress	(Cohen, Kamarch, & Mermelstein, 1983)[57]
Rape Stigma Scale (RSS)[1]	Nine items e.g. 'I have been concerned about people respecting me less if they were to find out what happened?'. Responses recorded on a 4-point Likert scale ranging from 1 ('never') to 4 ('many times'). Total score range between 9 and 36.	Baseline 3-months 6-months	.85	Rape stigma	(Kalichman et al., 2005)[58]
Alcohol Use Disorders Identification Test – Consumption Subscale (AUDIT-C)	Three items e.g. 'How many drinks containing alcohol do you have on a typical day when you are drinking?'. Responses recorded on a 5-point Likert scale with diverse response options dependent on the individual item. Total score range between 0 and 12. A score of 3 or more indicates hazardous alcohol use.	Baseline 3-months 6-months	.71	Alcohol consumption	(Saunders, Aasland, Babor, De La Fuente & Grant, 1993)[59]
Center for Epidemiologic	Twenty items e.g. 'I felt that I could not shake off the blues even with the help from my family or friends'. Responses	Baseline 3-months	.88	Depression	(Radloff, 1977)[60]

Studies Depression Scale (CES-D)	recorded on a 4-point Likert scale ranging from 0 ('rarely or none of the time') to 3 ('most or all of the time'). Total score range between 0 and 60.	6-months		
Context of Rape Questionnaire (CRQ)	Ten items e.g. 'How many men were involved in the rape?'. Diverse response options depending on the individual item.	Any timepoint except baseline[3]	(1) Number of men involved in the rape, (2) perpetrator known, (3) use of coercion, (4) perpetrator used a weapon, (5) bodily physical force/assault used, (6) perpetrator threatened to kill the survivor, (7) rape survivor thought they would be killed, (8) prior rape by the same perpetrator, (9) number of sexual acts (vaginal, anal, oral, digital or object penetration, forced observed masturbation), (10) rape reported to the police	(Jewkes et al., 2009)[61]

[1]Modified version of the original measure. [2]Response options were modified to reduce test fatigue and response errors by using a uniform response method across several measures.

Abbreviations: posttraumatic stress disorder (PTSD)

Data analysis

Pre-assault and post-assault variables

Most variables did not conform to a normal distribution and non-parametric statistics were therefore used in the univariate analyses prior to imputing missing values. Spearman's correlation coefficients were used to investigate the relationship between continuous variables and PTSD symptom total scores. Kruskal-Wallis, Mann-Whitney U and chi-square statistics were used to investigate the relationship between categorical variables and PTSD symptom scores.

Two sets of independent variables were included in the regression analysis to determine their relationship with the dependent variable 'PTSD symptom scores'. The first set of variables were defined as 'pre-assault variables' and were treated as time-independent variables. The pre-assault variables were further divided into pre-assault demographic factors and pre-assault trauma factors. Pre-assault demographic factors included the continuous variable age and the categorical variables education (secondary education completed vs not completed), employment status (employed vs unemployed), relationship status (in a relationship vs widowed/separated/divorced vs single) and HIV status (positive vs negative). Pre-assault trauma factors included the continuous variables childhood trauma and lifetime trauma.

The second set of variables were defined as 'post-assault variables' and were treated as time-dependent variables in the analysis. All post-assault variables were continuous variables and were further divided into protective factors and risk factors. Post-assault protective factors included resilience and social support and post-assault risk factors included perceived stress, rape stigma, alcohol use and depression. Alcohol scores were assessed in relation to the four weeks preceding the baseline visit, the time between the baseline visit and the 3-month visit and the time between the 3-month visit and the 6-month visits. Depression scores were assessed in relation to the week preceding the baseline, 3-month and 6-month visit.

Missing data and multiple imputation model

Six hundred and thirty-nine participants completed the baseline interview and 274 (42.9%) completed all three visits. Of the 639 participants, 368 (57.6%) completed the 3-month visit and 325 (50.9%) the 6-month visit. Two hundred and twenty-five participants (35.2%) completed the baseline visit only and as such did not have 3-month and 6-month data. One hundred and forty participants (21.9%) either missed the 3-month visit or 6-month visit.

Recording responses to the measures electronically ensured that there were no missing values at item level, given that the interviewer could not continue with the interview if responses were missing. However, an uploading error resulted in missing value sets for the symptom severity subscale of the DTS which was corrected once it was identified. There were no missing values for the DTS frequency subscale but 52.9% (n = 338) of the DTS symptom severity scores were missing at baseline, 46.2% (n = 127) were missing at 3-months post-rape and 43.4% (n = 141) were missing at 6-months post-rape. Missing DTS symptom severity values were imputed using a multiple imputation model while maintaining the multivariate normal distribution.

A comparison of participants who completed all visits, participants who had missed visits and those who only completed the baseline visit revealed significant group differences on baseline measures of childhood trauma (p = .026), resilience (p = .042), perceived stress (p = .002) and alcohol consumption (p = .003) (see Supplementary Table 1 for details). Missing values were treated as missing at random (MAR) and imputation of missing data was undertaken to reduce the bias introduced by data MAR [62].

Out of a potential 1917 observations (i.e. 639 completed interviews at baseline, 3-month and 6-month), 585 observations were missing (30.5%). A multiple imputation model using the Markov Chain Monte Carlo (MCMC) method was used to impute missing values at 3-months and 6-months post-rape for the dependent variable PTSD symptom total score and the time-dependent post-assault continuous variables. The missing values were imputed as a function of baseline scores for resilience, social support, perceived stress, rape stigma, alcohol use and depression as well as baseline demographic characteristics (age, education, relationship status, employment status, HIV status) and the baseline measure of childhood trauma and lifetime trauma. Thirty imputations were conducted, each time randomly selecting a data subset with complete values to predict the values of missing data while maintaining the participant specific correlation coefficient between the baseline time-independent and time-dependent measures and the time-dependent post-assault variables and PTSD symptom total score at 3-months and 6-months post-rape. The estimates of the 30 imputations were then pooled and the average value was used to impute the missing data. Imputed values were bounded according to the lowest and highest possible score in the range of the original measure.

Pre-assault and post-assault predictors of PTSD symptom severity and trajectory

We followed four steps to determine predictors of PTSD symptom scores and trajectory (visually presented in Figure 1). In all four steps we conducted the analysis based on imputed missing values. Firstly, we investigated baseline pre-assault variables, post-assault variables

and time (baseline, 3-months and 6-months) as predictors of PTSD symptom scores at 6-months post-rape in Model 1, using a mixed linear regression model.

Secondly, we again investigated time and the pre-assault and post-assault variables investigated in Model 1 but converted the pre-assault trauma variables and the post-assault risk and protective variables into categorical variables in Model 2, for ease of interpretation. Childhood trauma (physical/sexual/emotional abuse, neglect, domestic violence) scores were converted into four categories ranging from 1 ('no childhood trauma type endorsed') to 4 ('three or more childhood traumas types endorsed'). Lifetime trauma exposure was converted into five categories ranging from 1 ('no trauma endorsed') to 5 ('four or more traumas endorsed'). Alcohol consumption was converted into three categories, namely 1 ('no consumption'), 2 ('some consumption') and 3 ('hazardous consumption'). Resilience, social support, perceived stress, rape stigma and depression scores were divided into four quartiles each (see Table 2 for sub-group sizes and interquartile ranges).

Thirdly, we investigated the interaction between time (baseline, 3-months and 6-months post-rape) and the significant predictors of PTSD symptom scores resulting from Model 1 and Model 2. A mixed linear model was again used in this step (Model 3).

Fourthly, the least squares mean method was used to determine PTSD symptom scores at baseline, 3-months and 6-months post-rape while adjusting for the means of the significant predictor variables at baseline resulting from Model 1-3. The interaction between different quartile levels of the significant predictors and PTSD symptom scores at the three different timepoints were then plotted to visually present the effect of different levels of baseline predictors on PTSD symptom trajectory.

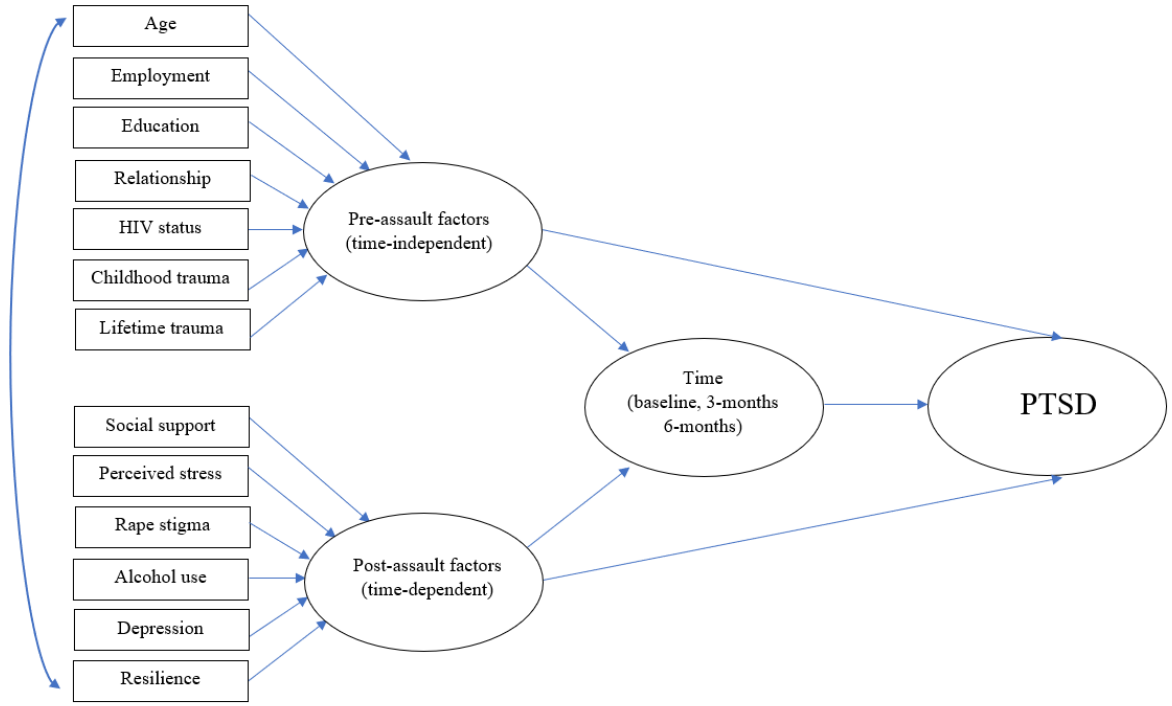


Figure 1: Pre- and post-assault variables by time as predictors of PTSD. Pre-assault and post-assault baseline predictors of PTSD were investigated in mixed linear regression Model 1 & Model 2. The interaction between baseline post-assault predictor variables and time in relation to PTSD symptom scores were investigated in mixed linear regression Model 3.

Assault variables and PTSD symptom scores

Assault variables included the number of men involved in the rape, perpetrator known to the rape survivor, use of coercion, perpetrator used a weapon, physical force or assault used, rape survivor threatened with death, rape survivor thought she would die, prior rape by the same perpetrator, number of different sexual acts and rape reported to the police (see Figure 2 for a detailed description of assault variables). All assault variables were categorical variables and were treated as time-dependent variables.

In the RICE study participants were followed-up beyond 6-months and assault variables were measured at any one of several timepoints (i.e. 3-, 6-, 12- or 18-months post-rape). The research team was advised by the police and the National Prosecuting Authority (NPA) not to ask questions pertaining to the rape if a participant had an ongoing legal case as disclosing information might influence the rape survivors testimony, compromise the case and reduce the likelihood of a successful conviction. This resulted in the availability of assault related data for a smaller subset of participants (n=168). We therefore did not include these variables in the mixed linear regression models but ran univariate analyses (Kruskal-Wallis, Mann-Whitney U

and chi-square statistics) to investigate the relationship between these variables and PTSD symptom scores at baseline, 3-months and 6-months post-rape (visually presented and variables defined in Figure 2).

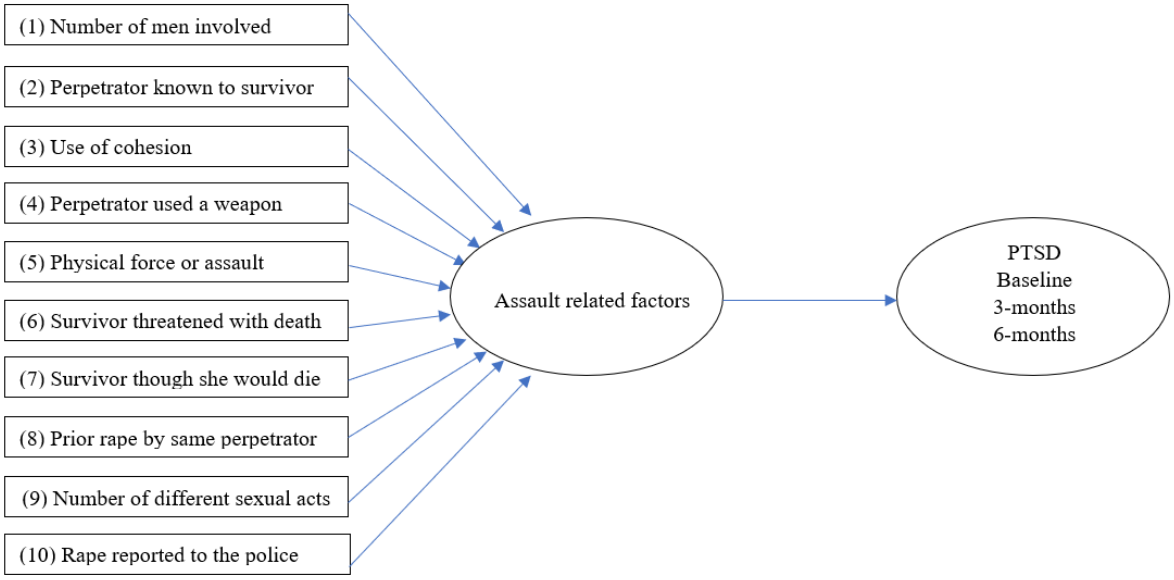


Figure 2: The relationship between assault variables and PTSD. Definition of assault variables: (1) the number of perpetrators and accomplices involved in the rape including those who had sexual contact with the rape survivor and/or lured, abducted, physically assaulted or verbally threatened the rape survivor or participated in any other way in the rape; (2) if the perpetrator was known to the rape survivor (acquaintance, friend, boyfriend, husband, family member, employer, teacher, priest, policemen etc.) or was a stranger; (3) if the rape survivor was abducted, lured somewhere under false pretences (e.g. a job offer, providing directions, offering transport etc.) or not; (4) if a weapon was used by the perpetrator (e.g. firearm, knife, sharp tool/instrument, blunt object, rope, wire, chain) or not; (5) if bodily physical force/assault was used by the perpetrator (e.g. strangulation, hit with fists/hands, kicked, held very tight or physical pressure use, hand used to cover mouth or nose) or not; (6) if the perpetrator or accomplice threatened to kill the rape survivor or not; (7) if the rape survivor thought she would be killed or not; (8) if the same perpetrator/s had raped the same survivor on a previous occasion/s; (9) the number of different sexual acts that occurred during the rape (vaginal/anal/oral/digital/object penetration, forced or observed masturbation); and (10) was the rape reported to the police or not.

RESULTS

PTSD symptom scores and incidence

Different timepoint mean (*M*) PTSD symptom scores and standard deviation (*SD*) were as follows: baseline (*M* = 68.1, *SD* = 24.7), 3-months (*M* = 39.3, *SD* = 24.1) and 6-months (*M* = 31.7, *SD* = 22.6). The incidence of PTSD at 3-months post-rape was 48.5% and 25.4% at 6-months post-rape (using a score of 40 or more on the DTS as indicative of the clinical threshold for PTSD).

Descriptive statistics of pre-assault and post-assault variables

Table 2 presents the descriptive statistics of the pre-assault and post-assault variables measured at baseline (n = 639). For pre-assault baseline demographic factors, the mean age of participants was 24.7 years (range 16-40), the majority of participants were married or in a relationship (80.8%), 57.6% completed secondary school (12 years of basic education), 78.4% were unemployed and 46.5% were HIV positive. For pre-assault trauma factors the mean number of childhood traumas reported was 1.3 (range 0-5) and the mean number of cumulative lifetime trauma exposures reported was 2.4 (range 0-10). For post-assault risk and protective factors at baseline, the mean scores were as follows: resilience (*M* = 74.5, *SD* = 6.3), social support (*M* = 35.0, *SD* = 5.0), perceived stress (*M* = 23.1, *SD* = 5.6), rape stigma (*M* = 20.7, *SD* = 6.9), alcohol use (*M* = 2.1, *SD* = 2.5) and depression (*M* = 33.0, *SD* = 12.6).

Table 2: *Descriptive statistics for baseline pre-assault and post-assault variables*

	n	%	*M(SD)*	Range
Pre-assault demographic factors				
Age	639	100	24.7(5.3)	16-40
Secondary education completed	639	100		
No	271	42.4		
Yes	368	57.6		
Relationship status	639	100		
In a relationship	516	80.8		
Widowed/separated/divorced	1	0.2		
Single	122	19.1		
Employment status	639	100		
Employed	138	21.6		
Unemployed	501	78.4		
HIV status	639	100		
Positive	297	46.5		
Negative	342	53.5		
Pre-assault trauma factors				
Number of childhood traumas (CTQ-SF)[1]	639	100	1.3(1.3)	0-5
No childhood trauma	244	38.2		
1 childhood trauma	155	24.3		
2 childhood traumas	121	18.9		
3 or more childhood traumas	119	18.6		
Number of lifetime traumas (LEC)[1]	639	100	2.4(1.5)	1-10
No lifetime trauma	119	18.6		
1 lifetime trauma	139	21.8		
2 lifetime traumas	161	25.2		
3 lifetime traumas	123	19.2		
4 or more lifetime traumas	97	15.2		
Post-assault protective factors				
Resilience (CD-RISC)[2]	639	100	74.5(6.3)	51-100
1st quartile	157	24.6	68.1(3.4)	0-71
2nd quartile	127	19.9	72.7(0.5)	72-73
3rd quartile	86	13.4	74.0(0.0)	74
4th quartile	269	42.1	79.3(6.2)	75-100
Social Support (MSPSS)[2]	639	100	35.0(5.0)	12-48
1st quartile	113	17.7	27.6(3.2)	0-31
2nd/3rd quartile	194	30.4	33.5(1.3)	32-35
4th quartile	332	52.9	38.3(3.6)	36-48
Post-assault risk factors				
Perceived stress (PSS)[2]	639	100	23.1(5.6)	10-40
1st quartile	148	23.2	15.4(2.1)	1-18
2nd quartile	136	21.3	21.0(1.1)	19-22
3rd quartile	193	30.2	24.7(1.1)	23-26
4th quartile	162	25.3	30.0(2.9)	27-40

Rape Stigma (RSS)	639	100	20.7(6.9)	9-36
1st quartile	136	21.3	11.0(1.9)	0-14
2nd quartile	162	25.4	17.5(1.8)	15-20
3rd quartile	172	26.9	23.0(1.4)	21-25
4th quartile	169	26.4	29.3(3.0)	26-36
Alcohol Use (AUDIT-C)	639	100	2.1(2.5)	0-12
No consumption	269	42.1	0.0(0.0)	0-0
Some consumption	152	23.8	1.5(0.5)	1-2
Hazardous consumption	218	34.1	2.0(0.1)	3-12
Depression (CES-D)	639	100	33.0(12.6)	0-60
1st quartile	154	24.1	15.5(6.6)	0-24
2nd quartile	165	25.8	29.7(2.9)	25-34
3rd quartile	155	24.3	38.0(2.1)	35-41
4th quartile	165	25.8	48.0(4.3)	42-60

[1]Modified version [2]Modified response option
Abbreviations: Mean (M); Standard Deviation (SD); Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test - Consumption (AUDIT-C); Center for Epidemiologic Studies Depression Scale (CES-D)

Univariate associations between PTSD and pre- and post-assault factors

PTSD symptom scores at one or more timepoint were associated with the pre-assault demographic variables employment and HIV status as well as the pre-assault trauma variables childhood trauma and lifetime trauma. Individual childhood trauma types associated with PTSD scores included neglect, domestic violence, emotional abuse, physical abuse and sexual abuse. Individual lifetime trauma types associated with PTSD scores included civil unrest/war, serious injury, being close to death, murder of a family member or friend and murder of a stranger. PTSD symptom scores at one or more timepoint were also associated with social support (putative protective factor post-assault) and perceived stress, rape stigma, alcohol use and depression (putative risk factors post-assault). See Supplementary Tables 2-4.

Baseline pre- and post-assault predictors of PTSD at 6-months post-rape

Table 3 presents the summary statistics of Model 1 investigating baseline pre-assault and post-assault factors as predictors of PTSD symptom severity at 6-months post-rape. The shared variance between the predictor variables significantly explained PTSD symptom severity at 6-months post-rape $F(16, 5020) = 42.2$, $p < .000$. PTSD symptom severity decreased significantly by 28.7 points from baseline to 3-months post-rape ($p < .001$) and by 36.4 points from baseline

to 6-months post-rape (p <.001). Baseline age, education, employment, relationship status, HIV status, childhood trauma, lifetime trauma, social support, resilience, perceived stress and alcohol use were not significant predictors of PTSD symptom severity at 6-months post-rape. Rape stigma ($ß$ = 0.72, p <.001) and depression ($ß$ = 0.63, p <.001) were the only significant predictors of PTSD symptom severity in this model. Model 2 followed the same design as Model 1, but the pre-assault trauma and post-assault putative risk and protective variables (i.e. continuous variables) were replaced by categorical variables. Rape stigma and depression were again the only variables significantly associated with PTSD symptom severity (see Supplementary Table 5 for details).

Table 3: *Summary statistics of the mixed linear regression model for investigating baseline pre-assault and post-assault predictors of PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
Time (baseline)[1]						
3-months post-rape	-28.74	1.88	-15.31	.000**	-32.46	-25.02
6-months post-rape	-36.37	1.85	-19.63	.000**	-40.02	-32.72
Age	-0.14	0.17	-0.80	.427	-.48	0.20
Basic education - not completed[1]						
Completed	0.21	1.66	0.12	.901	-3.60	3.47
Employment - unemployed[1]						
Employed	-1.57	2.01	-0.78	.436	-5.51	2.38
Relationship status - in a relationship[1]						
Separated/divorced/widow	-0.59	3.45	-0.17	.864	-7.38	6.19
Single	-1.44	2.04	-0.70	.483	-5.45	2.58
HIV status – negative[1]						
Positive	1.86	1.81	1.03	.304	-1.70	5.42
Childhood trauma (CTQ-SF)[2]	0.22	0.25	0.85	.393	-0.28	0.72
Lifetime trauma (LEC)[2]	0.61	0.48	1.29	.198	-0.32	1.55
Resilience (CD-RISC)[3]	0.06	0.15	0.41	.679	-.23	0.35
Social support (MSPSS)[3]	0.19	0.18	1.07	.285	-0.16	0.54
Perceived stress (PSS)[3]	0.11	0.16	0.70	.485	-0.21	0.43
Rape stigma (RSS)	0.72	0.14	5.30	.000**	0.45	0.99
Alcohol use (AUDIT-C)	0.15	0.34	0.45	.652	-0.52	0.82
Depression (CES-D)	0.63	0.07	8.84	.000**	0.49	0.77

[1] Reference categories in regression model [2]Modified version [3]Modified response options

Abbreviations: Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test – Consumption (AUDIT); Center for Epidemiologic Studies Depression Scale (CES-D)

Interaction between baseline depression, baseline rape stigma and PTSD symptom scores across time

Table 4 presents the summary statistics of Model 3 investigating the interaction effects for time x depression and time x rape stigma (significant predictors identified from Model 1 and Model 2) on PTSD symptom scores. The shared variance from the predictor variables and their interaction with time significantly explained PTSD symptom severity $F(20, 4969) = 35.6$, $p <$ .000 in the model as a whole. The interaction between time (for all timepoints) and rape stigma was not significant $F(6, 1367) = 1.61$, p =.140. However, when investigating individual timepoints participants with baseline rape stigma scores falling in the 3rd ($ß$ = -11.18, p =.029) and 4th ($ß$ = -15.36, p =.004) quartiles did show significantly increased PTSD symptom scores at 6-months post-rape compared to those with scores in the 1st quartile.

There was a significant interaction between time (for all timepoints) and depression $F(6, 1798) = 5.66$, $p <$.000. Participants with baseline depression scores falling in the 3rd ($ß$ = -17.81, $p <$.000) and 4th ($ß$ = -19.61, $p <$.000) quartiles had significantly increased PTSD symptom severity scores at 3-months post-rape compared to those falling in the 1st quartile. Participants with baseline depression scores falling in the 3rd ($ß$ = -19.01, $p <$.000) and 4th ($ß$ = -22.08, $p <$.000) quartile also had significantly increased PTSD symptom severity scores at 6-months post-rape compared to those falling in the 1st quartile.

Table 4: *Summary statistics of the mixed linear regression model investigating the interaction between time and baseline pre- and post-assault factors*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
Time (Baseline)						
3-months	-15.94	4.07	-3.91	.000**	-23.95	-7.93
6-months	-15.92	4.44	-3.58	.000**	-24.66	-7.18
Rape stigma (RSS) - 1st quartile[1]						
2nd quartile	8.62	3.17	2.72	.007**	2.39	14.84
3rd quartile	13.58	3.31	4.11	.000**	7.08	20.08
4th quartile	20.62	3.50	5.90	.000**	13.74	27.50
Rape stigma x Time - 1st quartile x baseline[1]						
2nd quartile x 3-months	-0.99	4.49	-0.22	.825	-9.81	7.83
2nd quartile x 6-months	-6.65	5.05	-1.32	.189	-16.60	3.30
3rd quartile x 3-months	-2.34	4.51	-0.52	.604	-11.22	6.53
3rd quartile x 6-months	-11.18	5.09	-2.20	.029*	-21.19	-1.16
4th quartile x 3-months	-5.16	5.14	-1.00	.317	-15.32	4.99
4th quartile x 6-months	-15.36	5.35	-2.87	.004**	-25.91	-4.82
Depression (CES-D) - 1st quartile[1]						
2nd quartile	11.93	3.15	3.79	.000**	5.74	18.12
3rd quartile	25.36	3.09	8.21	.000**	19.30	31.42
4th quartile	33.07	3.24	10.20	.000**	26.71	39.44
Depression x Time - 1st quartile x baseline[1]						
2nd quartile x 3-months	-4.62	4.20	-1.10	.271	-12.86	3.62
2nd quartile x 6-months	-5.43	4.71	-1.15	.250	-14.68	3.83
3rd quartile x 3-months	-17.81	4.34	-4.10	.000**	-26.33	-9.29
3rd quartile x 6-months	-19.01	4.88	-3.89	.000**	-28.62	-9.41
4th quartile x 3-months	-19.61	4.63	-4.24	.000**	-28.71	-10.50
4th quartile x 6-months	-22.08	4.87	-4.54	.000**	-31.64	-12.52

[1] Reference categories in regression model

Abbreviations: Rape Stigma Scale (RSS); Center for Epidemiologic Studies Depression Scale (CES-D)

Trajectories of depression, rape stigma and PTSD symptoms

Figure 3 illustrates the estimated mean PTSD symptom scores when considering the interaction between time and the depression quartiles at baseline (see Supplementary Table 6 for details). The PTSD symptom trajectories followed a similar pattern over time with a decline in PTSD symptoms visible for all depression quartiles. An upward shift in PTSD symptom severity over time corresponded to an upward shift in depression quartiles. Figures 4a-4d illustrate the estimated additive effect of falling in a higher rape stigma quartile at baseline on PTSD symptom trajectories by baseline depression quartiles.

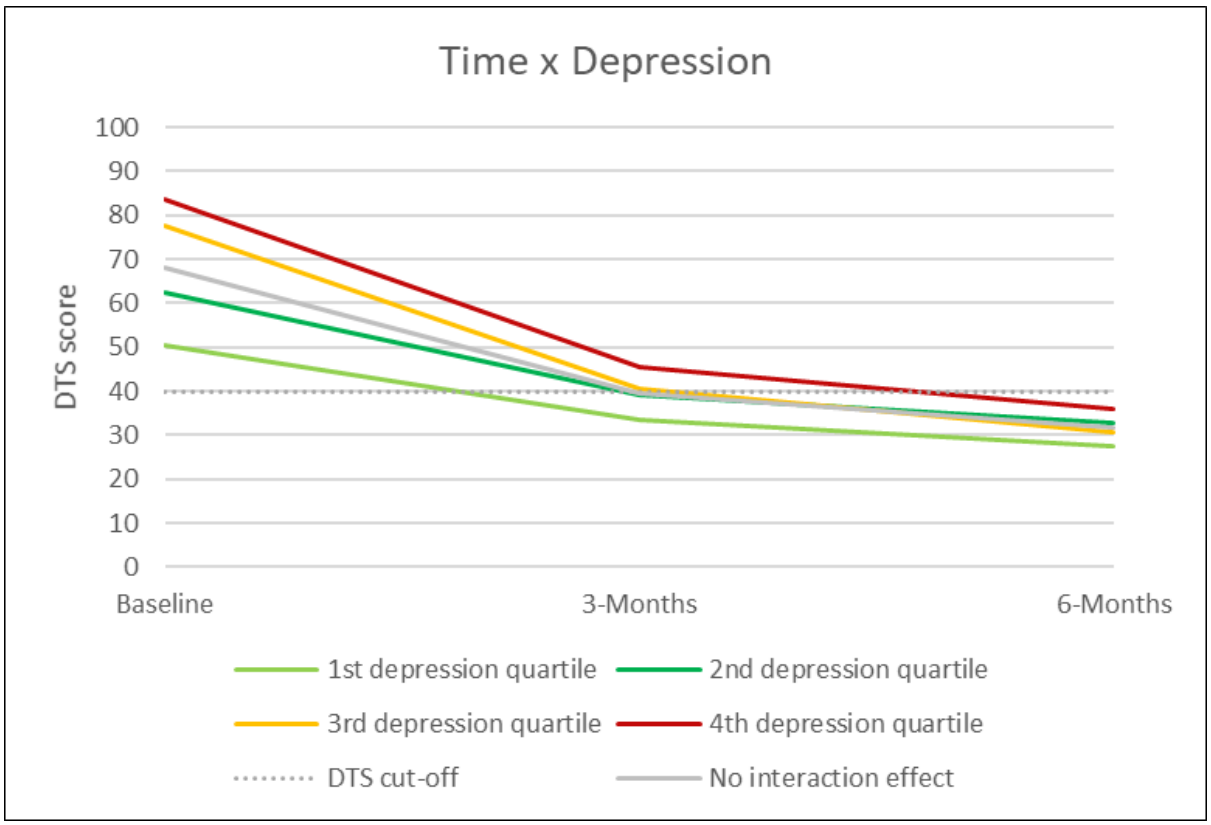


Figure 3: Time-specific effects of different depression quartiles at baseline on estimated PTSD symptom score trajectories. The *x*-axis presents time passed since the rape occurred, with baseline representing 0-20 days post-rape. The *y*-axis presents the PTSD symptom score measured with the Davidson Trauma Scale (DTS). The grey dotted line represents the DTS cut-off of 40 and the solid grey line represents the estimated mean PTSD symptom scores when not considering the interaction between time and depression quartiles.

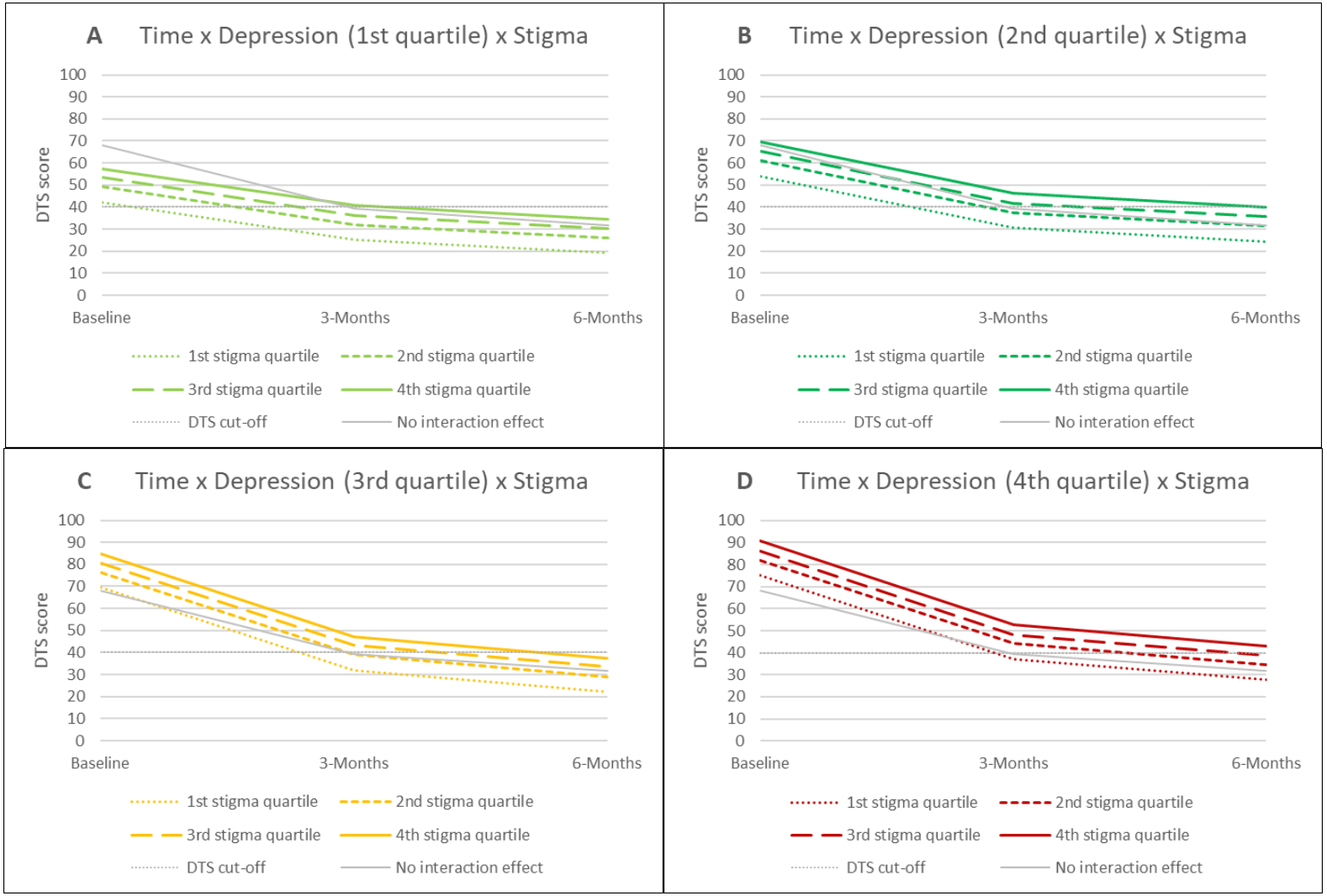


Figure 4: Time-specific effects of different depression quartiles with the added effect of different rape stigma quartiles at baseline on estimated PTSD symptom scores. The estimated mean PTSD trajectories when considering the 1st, 2nd, 3rd and 4th depression quartiles and the additional

effect of the rape stigma quartiles are consecutively presented in Figures 4a-4d. The *x*-axis presents the time passed since the rape occurred with baseline representing 0-20 days post-rape. The *y*-axis presents the PTSD symptom score measured with the Davidson Trauma Scale (DTS). The grey dotted line represents the DTS cut-off of 40 and the solid grey line represents the estimated mean PTSD symptom trajectory when not considering the interaction between time, depression quartiles and rape stigma quartiles.

Assault factors and PTSD symptom scores

Table 5 shows findings from the subset of participants who disclosed assault characteristics. None of the assault variables were significantly associated with PTSD symptom scores at any timepoint, with the exception of the number of men involved in the rape which was associated with PTSD symptom scores at 3-months post-rape (p = .032). Rape survivors reporting a single perpetrator had higher PTSD scores at 3-months post-rape (M = 43.9, SD = 29.4) compared to those who reported multiple perpetrators (M = 36.9, SD = 29.5).

Table 5: *Assault factors in relation to PTSD symptom scores over time*

	n(%)	M(SD)	Baseline PTSD score		3-month PTSD score		6-month PTSD score	
			M(SD)	*p*	M(SD)	*p*	M(SD)	*p*
Number of men involved in the rape	152(100)	1.3(1.0)		.676		.032*		.833
1 perpetrator	138(90.8)		70.9(25.2)		43.9(29.4)		31.0(28.8)	
More than 1 perpetrator	14(9.2)		68.0(21.6)		36.9(29.5)		30.3(24.3)	
Relationship to perpetrator	165(100)			.838		.559		.600
Known	114(69.1)		69.9(26.2)		41.8(29.1)		29.7(27.8)	
Not known	51(30.9)		71.4(21.5)		44.5(30.8)		30.9(27.6)	
Use of coercion	167(100)			.495		.116		.290
Abducted	55(32.9)		73.5(20.5)		36.5(26.8)		27.3(27.2)	
Lured under false pretences	26(15.6)		67.6(21.0)		50.4(32.1)		37.1(31.4)	
Not abducted	86(51.5)		69.1(27.8)		44.9(30.0)		30.3(27.8)	
Perpetrator used a weapon	168(100)			.363		.718		.526
Yes	51(30.4)		73.6(20.4)		41.3(27.6)		30.5(28.6)	
No	85(50.6)		69.5(26.5)		42.8(30.2)		29.2(27.6)	
Unsure	32(19.0)		66.3(25.3)		46.8(31.2)		34.7(28.6)	
Bodily physical force/assault used	160(100)			.674		.492		.441
Yes	134(83.8)		70.0(23.3)		43.1(29.6)		30.4(29.1)	
No	26(16.2)		68.4(31.0)		37.8(26.1)		31.7(23.5)	
Perpetrator threatened to kill the survivor	163(100)			.397		.932		.699
Yes	75(46.0)		72.7(21.0)		42.6(32.0)		28.3(26.0)	
No	88(54.0)		68.6(27.6)		41.6(26.3)		31.4(29.4)	
Rape survivor thought she would be killed	164(100)			.763		.127		.175
Yes	114(69.5)		71.2(23.1)		44.8(30.5)		24.4(22.1)	
No	50(30.5)		69.0(28.4)		36.3(24.2)		33.2(29.9)	
Prior rape by the same perpetrator	168(100)			.699		.332		.905
Yes	15(8.9)		68.9(20.6)		34.9(23.3)		27.7(20.1)	
No	153(91.1)		70.3(25.0)		43.9(30.0)		30.9(28.7)	

Number of sexual acts (vaginal, anal, oral, digital or object penetration, forced or observed masturbation)	160(100)	1.9(1.6)		.253		.226		.272
1 act	97(60.6)		68.0(26.0)		40.3(29.2)		29.3(29.4)	
2 or more acts	63(39.4)		72.8(22.1)		46.0(29.5)		30.6(28.6)	
Reported to police	169(100)			.199		.716		.753
Yes	139(82.3)		71.6(22.9)		42.6(29.3)		30.8(28.8)	
No	30(17.7)		64.1(30.8)		45.1(30.5)		30.3(24.2)	

Abbreviations: Mean (M), Standard Deviation (SD)

DISCUSSION

Consistent with previous longitudinal studies, we found a high incidence of PTSD at 3-months post-rape. Previous studies reported that 35% - 45% of rape and/or sexual assault survivors met criteria for PTSD at 3-months post-rape, we found that 48,5% of the sample scored above the clinical cut-off for PTSD in this sample [9,17–19]. We also found that 25.4% of participants remained above the clinical cut-off for PTSD at 6-months post-rape which is consistent with literature highlighting the long-term adverse effects of rape on mental health [18,19,23]

Pre-assault demographic factors age, education, employment, relationship status, HIV status, childhood trauma exposure and cumulative lifetime trauma exposure were *not* significant predictors of PTSD in the multivariate analyses. Most previous studies of risk factors for PTSD after rape have also not found a significant association between socio-demographic factors and PTSD [9,18,26,28,33,63]. Pre-assault childhood trauma and cumulative lifetime trauma were found to be strong predictors of PTSD in samples of participants with a mix of index trauma types [44,46,64] but inconsistent findings have been reported for rape and sexual assault and some support the non-significant findings found in the multivariate analyses in our study [9,18,26–28,63].

Depression and rape stigma post-assault were the only significant predictors of PTSD, with depression being the strongest predictor. Previous studies on rape and sexual assault have also reported a link between higher baseline depression scores and increased PTSD symptom scores over time [18,27,63]. Depression may be viewed as a comorbid condition that emerges following trauma exposure as many individuals with PTSD are likely to meet criteria for depression as well [65–67]. One longitudinal study investigating sexual assault survivors found that PTSD symptom severity fully mediated the relationship between depression symptom severity and the effect of time [19]. The study therefore suggests that a decrease in depression symptoms over time is directly linked to a decrease in PTSD symptoms and that depression likely develops secondary to PTSD following sexual assault [19]. Some of the symptoms of depression also overlaps with the symptoms of PTSD (e.g. negative alterations in cognitions and mood and alteration in arousal and reactivity)[68]. This overlap may partly explain the significant association between PTSD symptom scores and depression scores reported in this study [65–67]. A higher rate of lifetime depression has also been associated with PTSD in two longitudinal sexual assault studies and suggests that premorbid depression may be a risk factor for the development of PTSD following sexual assault [18,34]. We are not able to deduce from

our findings that high depression scores post-rape were the result of rape since we did not take a prior history of depression into account in the analysis.

The significant relationship between rape stigma and PTSD corresponds to findings of several prior studies investigating sexual assault where negative social reactions were associated with increased PTSD symptom severity [10,25,26,31]. Rape stigma, in this study and previous studies, is mostly defined by negative, morally rooted emotions such as self-blame, embarrassment and shame, disrespect and avoidance from others and the belief that the victim should have done more to prevent the rape [10,69,70]. Rape stigma and social support seem to be closely interrelated with increased rape stigma associated with a decreased ability to access and rely on social support [10,45,69]. Since rape is a violation at an interpersonal level, it is plausible that victims develop a sense of distrust in others and when this distrust is not repaired over time through positive supportive responses, this may result in more severe emotional distress [9,27]. Decreased social support and increased self-blaming is also likely to hinder treatment seeking and long-term recovery [9]. The majority of rape survivors in this study were acquainted with the perpetrator. This has also been found to contribute to others blaming the victim and holding the victim responsible for the event; this is especially true when the perpetrator is a family member or a respected member of the community [10,25,31,45,47].

It has been suggested that the aggressive act of rape and its intrusive nature can result in such severe distress that it overrides the risk and protective factors that are generally associated with PTSD [9,71,72]. This may explain the non-significant relationship found between resilience, social support and PTSD in the multivariate analyses in this study. Depression and rape stigma may also inhibit the role of protective factors such as resilience and social support to such a degree that the variance explained by the latter may be masked by the variance explained by the former.

Similar to the findings of a recent longitudinal study in survivors of completed and attempted rape, we did not find that alcohol consumption significantly contributed to PTSD scores [18]. Perceived stress was also not associated with PTSD. It should be noted that the majority of longitudinal sexual assault studies have investigated sexual assault in general and not rape or completed rape exclusively [9,18,19,38]. In this study we investigated survivors of rape only. Rape compared to non-penetrative sexual assault may have further exacerbated emotional distress especially if it was accompanied by physical violence or threats of violence [18,25,26,30]. Differences in the type of sexual assault investigated in previous studies may explain inconsistencies between our finding and prior findings.

None of the assault-related factors were associated with PTSD at any timepoint, with the exception of number of perpetrators/accomplices involved in the rape. Previous studies investigating assault-related variables have reported inconsistent findings [9,18,25,26,63]. Some studies have found a significant relationship between PTSD and multiple perpetrators [18], multiple sexual acts [18], physical injury inflicted during the rape [18,25] longer duration of assault [30] and perceived life threat [25,26,30] while other studies did not [9,63]. We found that rape involving a single perpetrator was associated with higher PTSD scores at 3-months post-rape compared to rape involving multiple perpetrators. This finding is counterintuitive and should be interpreted with caution given that (1) the assault-related variables was investigated using univariate analysis only and the findings were not corrected for multiple testing and (2) only a sub-sample of participants disclosed this information and the reasons for non-disclosure (e.g. emotionally unable to disclose, ongoing legal case) may themselves be associated with PTSD symptom scores and may have introduced bias.

In conclusion, we found that depression and rape-stigma had a significant adverse impact on the PTSD recovery trajectory following rape. Identifying risk factors present before trauma exposure and manifesting soon after a traumatic event could potentially guide early interventions and reduce the number of PTSD cases resulting from rape and sexual violence [18,23]. Targeted early interventions are especially important in low- and middle-income countries characterised by limited resources and overburdened mental health care facilities since treatment of severe psychopathology is more resource intensive (e.g. longer duration of treatment, medical interventions, hospitalisation, management of comorbid disorders) with higher costs and burden for the healthcare system [73,74]. Brief interventions, including psychoeducation, targeting cognitive distortions and stigma around rape, could be delivered by trained counsellors attending to those reporting the rape for the first time. Depression should also be assessed in rape survivors and those presenting with high levels of depression at rape crisis centres and/or hospitals should be appropriately referred and managed accordingly.

Strengths and limitations

Some limitations were unavoidable in this study and deserve mention. All participants disclosed the rape since they all sought help from rape crisis centres before enrolling in the study. As such, we could not assess risk and protective factors associated with PTSD in non-disclosing rape survivors. Fear of contracting HIV may have contributed to emotional distress, but this was not considered in the analyses. Some participants received counselling at the rape crisis centres at which they presented and/or at the study site. We could not include counselling

as a predictor of PTSD scores in the multivariate analyses since we did not have this information for those who were lost to follow-up. Current psychotropic and psychotherapeutic interventions which may influence PTSD symptom severity were not considered as covariates. We could not include assault-related variables in the multivariate analyses since they were reported by a small sub-set of participants only. Lastly, the self-report measure of perceived stress showed poor reliability in the sample and the results related to this measure should be interpreted with caution.

This study also had several strengths. First, the longitudinal design permitted causal and consequential inferences about risk and protective factors for PTSD in a large cohort of rape-exposed women to be made. Second, all women in this study were exposed to a homogenous trauma (i.e. rape), in contrast to most previous longitudinal studies that have lumped sexual assault, rape and attempted rape together. Third, the study was adequately powered to investigate a variety of risk and protective factors that have previously been documented to be associated with PTSD in mixed trauma samples. Finally, we did not exclude participants with missing data but imputed missing values using a multiple imputation model which limits bias caused by drop-out and other associated factors. We also used a mixed linear regression model which allowed us to consider individual symptom trajectories over time.

REFERENCES

1. Statistics South Africa. *Governance, Public Safety and Justice Survey: GPSJS 2018/2019*. Pretoria, South Africa; 2019.

2. Wilkinson K. *Rape Statistics in South Africa*. Johannesburg, South Africa; 2018. https://africacheck.org/factsheets/guide-rape-statistics-in-south-africa/.

3. Jewkes R, Abrahams N. The epidemiology of rape and sexual coercion in South Africa: An overview. *Soc Sci Med*. 2002;55(7):1231-1244. doi:10.1016/S0277-9536(01)00242-8

4. Machisa M, Jewkes R, Morna C, Rama K. *The War at Home*. Johannesburg, South Africa; 2011.

5. Kleber R. Trauma and Public Mental Health : A Focused Review. *Front Psychiatry*. 2019;10:1-6. doi:10.3389/fpsyt.2019.00451

6. Maercker A, Hecker T. Broadening perspectives on trauma and recovery: † a socio-interpersonal view of PTSD. 2016;8198. doi:10.3402/ejpt.v7.29303

7. Santiago PN, Ursano RJ, Gray CL, et al. A Systematic Review of PTSD Prevalence and Trajectories in DSM-5 Defined Trauma Exposed Populations : Intentional and Non-Intentional Traumatic Events. *PLoS One*. 2013;8(4):e59236. doi:10.1371/journal.pone.0059236

8. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

9. Elklit A, Christiansen DM. Risk factors for posttraumatic stress disorder in female help-seeking victims of sexual assault. *Violence Vict*. 2013;28(3):552-568. doi:10.1891/0886-6708.09-135

10. Moor A, Farchi M. Is rape-related self blame distinct from other post traumatic attributions of blame? A comparison of severity and implications for treatment. *Women Ther*. 2011;34(4):447-460. doi:10.1080/02703149.2011.591671

11. Breslau N. The epidemiology of posttraumatic stress disorder: What is the extent of the

problem? *J Clin Psychiatry*. 2001;62(17):16-22.

12. Hetzel-Riggin MD, Roby RP. Trauma Type and Gender Effects on PTSD, General Distress, and Peritraumatic Dissociation. *J Loss Trauma*. 2013;18(1):41-53. doi:10.1080/15325024.2012.679119

13. Kolltveit S, Lange-Nielsen II, Thabet AAM, et al. Risk factors for PTSD, anxiety, and depression among adolescents in Gaza. *J Trauma Stress*. 2012;25(2):164-170. doi:10.1002/jts.21680

14. Meewisse M-L, Reitsma JB, De Vries G-J, Gersons BPR, Olff M. Cortisol and post-traumatic stress disorder in adults. *Br J Psychiatry*. 2007;191(5):387-392. doi:10.1192/bjp.bp.106.024877

15. Stein MB, Kennedy C. Major depressive and post-traumatic stress disorder comorbidity in female victims of intimate partner violence. *J Affect Disord*. 2001;66(2-3):133-138. doi:10.1016/S0165-0327(00)00301-3

16. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: A quantitative review of 25 years of research. *Psychol Bull*. 2006;132(6):959-992. doi:10.1037/0033-2909.132.6.959

17. Elklit A, Christiansen DM. ASD and PTSD in rape victims. *J Interpers Violence*. 2010;25(8):1470-1488. doi:10.1177/0886260509354587

18. Möller AT, Bäckström T, Söndergaard HP, Helström L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One*. 2014;9(10):1-9. doi:10.1371/journal.pone.0111136

19. Nickerson A, Steenkamp M, Aerka IM, et al. Prospective investigation of mental health following sexual assault. *Depress Anxiety*. 2013;30(5):444-450. doi:10.1002/da.22023

20. Atwoli L, Stein DJ, Williams DR, Mclaughlin KA, Petukhova M, Kessler RC. Trauma and posttraumatic stress disorder in South Africa : analysis from the South African Stress and Health Study. *BMC Psychiatry*. 2013;13(182).

21. Breslau N, Troost JP, Bohnert K, Luo Z. Influence of predispositions on post-traumatic stress disorder: Does it vary by trauma severity? *Psychol Med*. 2013;43(2):381-390.

doi:10.1017/S0033291712001195

22. Yehuda R, Mcfarlane A, Vermetten E, Lanius R. Post-traumatic stress disorder. *Nat Rev*. 2015;1(October):1-22. doi:10.1038/nrdp.2015.57

23. Campbell R, Sprague HB, Cottrill S, Sullivan CM. Longitudinal research with sexual assault survivors: A methodological review. *J Interpers Violence*. 2011;26(3):433-461. doi:10.1177/0886260510363424

24. Campbell R, Adams AE, Wasco SM, Ahrens CE, Sefl T. "What has it been like for you to talk with me today?": The impact of participating in interview research on rape survivors. *Violence Against Women*. 2010;16(1):60-83. doi:10.1177/1077801209353576

25. Ullman S, Filipas H. Predictors of PTSD symptom severity and social reactions in sexual assault victims. *J Trauma Stress*. 2001;14(2):369-389. doi:10.1023/A

26. Ullman S, Filipas H, Townsend S, Starzynski L. Psychosocial correlates of PTSD symptom severity in sexual assault survivors. *J Trauma Stress*. 2007;20(5):821-831. doi:10.1002/jts.20290

27. Bryant-Davis T, Ullman SE, Tsong Y, Tillman S, Smith K. Struggling to survive: Sexual assault, poverty, and mental health outcomes of african american women. *Am J Orthopsychiatry*. 2010;80(1):61-70. doi:10.1111/j.1939-0025.2010.01007.x

28. Nishith P, Mechanic MB, Resick PA. Prior interpersonal trauma: The contribution to current PTSD symptoms in female rape victims. *J Abnorm Psychol*. 2000;109(1):20-25. doi:10.1037/0021-843X.109.1.20

29. Machtinger EL, Wilson TC, Haberer JE, Weiss DS. Psychological trauma and PTSD in HIV-positive women: A meta-analysis. *AIDS Behav*. 2012;16(8):2091-2100. doi:10.1007/s10461-011-0127-4

30. Kaysen D, Rosen G, Bowman M, Resick PA. Duration of Exposure and the Dose-Response Model of PTSD. *J Interpers Violence*. 2010;25(1):63-74. doi:10.1177/0886260508329131

31. Ullman SE, Filipas HH, Townsend SM, Starzynski LL. The role of victim-offender relationship in women's sexual assault experiences. *J Interpers Violence*.

2006;21(6):798-819. doi:10.1177/0886260506288590

32. Kessler R, Rose S, Koenen K, et al. How well can post-traumatic stress disorder be predicted from pre-trauma risk factors ? An exploratory study in the WHO World Mental Health Surveys. *World Psychiatry*. 2014;13:265-274.

33. Bryant-Davis T, Ullman SE, Tsong Y, Gobin R. Surviving the storm: The role of social support and religious coping in sexual assault recovery of African American women. *Violence Against Women*. 2011;17(12):1601-1618. doi:10.1177/1077801211436138

34. Breslau N, Davis GC, Peterson EL, Schultz LR. A second look at comorbidity in victims of trauma: The posttraumatic stress disorder-major depression connection. *Biol Psychiatry*. 2000;48(9):902-909. doi:10.1016/S0006-3223(00)00933-1

35. Resnick HS, Walsh K, Schumacher JA, Kilpatrick DG, Acierno R. Prior substance abuse and related treatment history reported by recent victims of sexual assault. *Addict Behav*. 2013;38(4):2074-2079. doi:10.1016/j.addbeh.2012.12.010

36. Resnick HS, Walsh K, McCauley JL, Schumacher JA, Kilpatrick DG, Acierno RE. Assault related substance use as a predictor of substance use over time within a sample of recent victims of sexual assault. *Addict Behav*. 2012;37(8):914-921. doi:10.1016/j.addbeh.2012.03.017

37. Testa M, Livingston JA, Hoffman JH. Does sexual victimization predict subsequent alcohol consumption? A prospective study among a community sample of women. *Addict Behav*. 2007;32(12):2926-2939. doi:10.1016/j.addbeh.2007.05.017

38. Kaysen D, Neighbors C, Martell J, Fossos N, Larimer ME. Incapacitated rape and alcohol use: A prospective analysis. *Addict Behav*. 2006;31(10):1820-1832. doi:10.1016/j.addbeh.2005.12.025

39. Kilpatrick DG, Ruggiero KJ, Acierno R, Saunders BE, Resnick HS, Best CL. Violence and risk of PTSD, major depression, substance abuse/dependence, and comorbidity: Results from the national survey of adolescents. *J Consult Clin Psychol*. 2003;71(4):692-700. doi:10.1037/0022-006X.71.4.692

40. Simpson TL, Miller WR. Concomitance between childhood sexual and physical abuse

and substance use problems: A review. *Clin Psychol Rev*. 2002;22(1):27-77. doi:10.1016/S0272-7358(00)00088-X

41. Logan T, Walker R, Cole J, Leukefeld C. Victimization and Substance Abuse among Women: Contributing Factors, Interventions, and Implications. *Rev Gen Psychol*. 2002;6(4):325-397. doi:10.1037/1089-2680.6.4.325

42. Klest B. Childhood trauma, poverty, and adult victimization. *Psychol Trauma Theory, Res Pract Policy*. 2012;4(3):245-251. doi:10.1037/a0024468

43. Lilly MM, London MJ, Bridgett DJ. Using SEM to examine emotion regulation and revictimization in predicting PTSD symptoms among childhood abuse survivors. *Psychol Trauma Theory, Res Pract Policy*. 2014;6(6):644-651. doi:10.1037/a0036460

44. Weich S, Patterson J, Shaw R, Stewart-Brown S. Family relationships in childhood and common psychiatric disorders in later life: systematic review of prospective studies. *Br J Psychiatry*. 2009;194(5):392-398. doi:10.1192/bjp.bp.107.042515

45. Wasco SM. Conceptualizing the Harm done by Rape: Applications of Trauma Theory to Experiences of Sexual Assault. *Trauma, Violence, Abus*. 2003;4(4):309-322. doi:10.1177/1524838003256560

46. Flouri E. Post-Traumatic Stress Disorder (PTSD): What we have learned and what we still have not found out. *J Interpers Violence*. 2005;20(4):373-379. doi:10.1177/0886260504267549

47. Carretta CM, Burgess AW, DeMarco R. To Tell or Not to Tell. *Violence Against Women*. 2016;22(13):1499-1518. doi:10.1177/1077801215627507

48. Abrahams N, Jewkes R, Mathews S. Depressive symptoms after a sexual assault among women: understanding victim-perpetrator relationships and the role of social perceptions. *Afr J Psychiatry*. 2013;16(4):288-293. doi:10.4314/ajpsy.v16i4.39

49. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296

50. Jewkes R, Nduna M, Levin J, et al. A cluster randomized-controlled trial to determine the effectiveness of Stepping Stones in preventing HIV infections and promoting safer

sexual behaviour amongst youth in the rural Eastern Cape, South Africa: Trial design, methods and baseline findings. *Trop Med Int Heal*. 2006;11(1):3-16. doi:10.1111/j.1365-3156.2005.01530.x

51. Sheehan D, Janacs J, Baker R, Sheehan K, Knapp E, Sheehan M. Mini international neuropsychiatric interview - version 7.0.0 DSM-5. http://www.medical-outcomes.com. Published 2014. Accessed July 2, 2015.

52. Bernstein D, Fink L. *Childhood Trauma Questionnaire: A Retrospective Self-Report Manual*. San Antonio: The Psychological Corporation; 1998.

53. Weathers F, Blake D, Schnurr P, Kaloupek D, Marx B, Keane T. *The Life Events Checklist for DSM-5 (LEC-5)*. USA: National Center for PTSD; 2013.

54. Davidson JRT, Book SW, Colket JT, et al. Assessment of a new self-rating scale for post-traumatic stress disorder. *Psychol Med*. 1997;27(1):153-160. doi:10.1017/S0033291796004229

55. Connor KM, Davidson JRT. Development of a new Resilience scale: The Connor-Davidson Resilience scale (CD-RISC). *Depress Anxiety*. 2003;18(2):76-82. doi:10.1002/da.10113

56. Zimet GD, Dahlem NW, Zimet SG, Farley GK. The Multidimensional Scale of Perceived Social Support. *J Pers Assess*. 1988;52(1):30-41. doi:10.1207/s15327752jpa5201_2

57. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24(4):385-396.

58. Kalichman SC, Simbayi LC, Jooste S, et al. Development of a brief scale to measure AIDS-related stigma in South Africa. *AIDS Behav*. 2005;9(2):135-143. doi:10.1007/s10461-005-3895-x

59. Saunders J, Aasland O, Babor T, De La Fuente J, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction*. 1993;88(6):791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

60. Radloff L. The CES-D Scale: A Self-Report Depression Scale for Research in the

General Population. *Appl Psychol Meas*. 1977;1(3):385-401. doi:10.1002/ardp.19552881111

61. Jewkes R, Christofides N, Vetten L, Jina R, Sigsworth R, Loots L. Medico-legal findings, legal case progression, and outcomes in South African rape cases: Retrospective review. *PLoS Med*. 2009;6(10). doi:10.1371/journal.pmed.1000164

62. Jakobsen JC, Gluud C, Wetterslev J, Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials - A practical guide with flowcharts. *BMC Med Res Methodol*. 2017;17(1):1-10. doi:10.1186/s12874-017-0442-1

63. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

64. Cloitre M, Stolbach B, Herman J, et al. A developmental approach to complex PTSD: childhood and adult cumulative trauma as predictors of symptom complexity. *J Trauma Stress*. 2009;22(5):293-300. doi:10.1002/jts

65. Flory J, Yehuda R. Comorbidity between post-traumatic stress disorder and major depressive disorder: alternative explanations and treatment considerations. *Dialogues Clin Neurosci*. 2015;17(2):141-150.

66. Spinhoven P, Penninx BW, Hemert AM Van, Rooij M De, Elzinga BM. Child Abuse & Neglect Comorbidity of PTSD in anxiety and depressive disorders : Prevalence and shared risk factors &. *Child Abuse Negl*. 2014;38(8):1320-1330. doi:10.1016/j.chiabu.2014.01.017

67. Greene T, Neria Y, Gross R. Prevalence , Detection and Correlates of PTSD in the Primary Care Setting : A Systematic Review. *J Clin Psychol Med Settings*. 2016;23(2):160-180. doi:10.1007/s10880-016-9449-8

68. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, USA; 2013.

69. Carretta CM, Burgess AW, DeMarco R. To tell or not to tell. [References]. *Violence Against Women*. 2016;21(9):1145-1165. doi:10.1177/1077801215590672

70. Starzynski LL, Ullman SE, Filipas HH, Townsend SM. Correlates of women's sexual assault disclosure to informal and formal support sources. *Violence Vict*. 2005;20(4):417-432. doi:10.1891/0886-6708.20.4.417

71. Dancu C V., Riggs DS, Hearst-Ikeda D, Shoyer BG, Foa EB. Dissociative experiences and posttraumatic stress disorder among female victims of criminal assault and rape. *J Trauma Stress*. 1996;9(2):253-267. doi:10.1007/BF02110659

72. Kessler R, Sonnega A, Bromet E, Hughes M, Nelson C. Posttraumatic stress disorder in the National Comorbidity survey. *Arch Gen Psychiatry*. 1995;52:1048-1060.

73. Kearns M, Ressler K, Zatzick D, Rothbaum B. Early interventions for ptsd: a review. *Depress Anxiety*. 2012;842:833-842. doi:10.1002/da.21997

74. Tol WA, Barbui C, Bisson J, et al. World Health Organization Guidelines for Management of Acute Stress , PTSD , and Bereavement : Key Challenges on the Road Ahead. *PLOS Med*. 2014;11(12):e1001769. doi:10.1371/journal.pmed.1001769

CHAPTER 4

GENOME-WIDE DIFFERENTIALLY METHYLATED GENES ASSOCIATED WITH POSTTRAUMATIC STRESS DISORDER AND LONGITUDINAL CHANGE IN METHYLATION IN RAPE SURVIVORS

ABSTRACT

Introduction: Rape is associated with a high risk for posttraumatic stress disorder (PTSD). DNA methylation changes may confer risk or protection for PTSD following rape by regulating expression of genes implicated in pathways affected by PTSD. We aimed to: (1) identify epigenome-wide differences in methylation profiles between rape-exposed women with and without PTSD at 3-months post-rape, in a demographically and ethnically similar group, drawn from a low-income setting; (2) validating and replicate the findings resulting from the epigenome-wide analysis in selected genes (*BRSK2* and *ADCYAP1*) at 3-months post-rape; and (3) investigate baseline and longitudinal changes in *BRSK2* and *ADCYAP1* methylation over six months in relation to change in PTSD symptom scores over 6 months, in the combined set. **Methods:** Rape-exposed women (n = 852) were recruited from rape clinics in the Rape Impact Cohort Evaluation (RICE) umbrella study. Epigenome-wide differentially methylated CpG sites between rape-exposed women with (n = 24) and without (n = 24) PTSD at 3-months post-rape were investigated using the Illumina MethylationEpic BeadChip, in a discovery cohort (n = 48). Validation (n = 48) and replication (n = 49) of the findings related to *BRSK2* and *ADCYAP1* methylation were investigated using EpiTYPER technology. Longitudinal change in *BRSK2* and *ADCYAP1* were also investigated using EpiTYPER technology in the combined set (n = 96). **Results:** One differentially methylated CpG site (chr10: 61385771/ cg01700569, adjusted for multiple comparisons p = .049) and thirty-four differentially methylated regions were associated with PTSD status at 3-months post-rape, in the discovery set. Decreased *BRSK2* CpG3 methylation at 3-months post-rape was associated with increased PTSD scores at 3-months post-rape. **Conclusion:** Decreased methylation of *BRSK2* may result in abnormal neuronal polarisation, synaptic development, vesicle formation and disrupted neurotransmission in individuals with PTSD at 3-months post-rape. PTSD symptoms may also be mediated by differential methylation of the *ADCYAP1* gene, which is involved in regulating the stress response. Replication of these findings are required to provide support for *ADCYAP1* and *BRSK2* as biomarkers and potential therapeutic targets of PTSD.

INTRODUCTION

Rape and sexual assault are associated with a high risk for the development of posttraumatic stress disorder (PTSD) compared to other trauma types [1,2]. Prospective studies have reported PTSD prevalence rates ranging between 35% and 45% at 3-months post-rape, with many survivors of sexual assault continuing to experience PTSD symptoms at 6- and 12-months post-rape [3–6]. PTSD is a complex, multifactorial disorder and an array of environmental and genetic putative risk and protective factors mediate or contribute to the development of the disorder [3,5,7]. Epigenetic mechanisms, including DNA methylation, are known to respond to environmental exposures such as trauma, leading to stable changes in gene expression [8,9]. DNA methylation responses may confer risk or protection for PTSD, as they may alter the ability to adapt to traumatic events on a molecular level [10]. Using a hypothesis-neutral, genome-wide approach to study epigenome-wide signatures (while accounting for potential environmental and biological confounding factors) and validating and replicating these findings, may bring us closer to uncovering the complexity of the disorder [10].

To date, twelve epigenome-wide association studies (EWASs) of blood DNA methylation differences in PTSD cases and controls have been published (see Table 1 for details). In sum, the majority of genes identified as differentially methylated in PTSD are linked to central nervous system functioning (e.g. neuron development, axonal outgrowth, synaptic connectivity, neurotransmitter release, neuroinflammation and apoptosis) [11–17] and the immune response (T-cell expression, cytokine and interferon release, phagocytosis) [13,14,18,19].

A meta-analysis of three North American mixed-gender civilian EWASs [13,17,18,20] found that PTSD was associated with the neuregulin1 *(NRG1)* and hepatocyte growth factor-regulated tyrosine kinase substrate *(HGS)*, both of which are related to central nervous system functioning [21]. The largest EWAS meta-analysis to date included 796 participants with PTSD and 1100 healthy controls [22]. North American and European male and female participants drawn from three civilian cohorts [13,17,18,20] and seven combat-exposed cohorts [15,16] were included. Associations with PTSD were observed at four CpG sites of the human aryl hydrocarbon receptor repressor *(AHRR)* gene, which has been linked to both pro- and anti-inflammatory immune regulation [22,23]. Ring finger protein 6 *(RNF6)*, also associated with immune function; ATPase phospholipid transporting 9A *(ATP9A)*, associated with glucose metabolism; family with sequence similarity 75, member D1 *(FLJ46321)*, associated with cell regulation; the microRNA 3170 *(MIR3170)*; and the long intergenic non-protein coding RNA 599 *(LINC00599)* genes were also associated with PTSD [22].

None of the gene-specific findings have been replicated across EWASs. Heterogeneity between and within EWASs may explain the lack of consistent findings. The majority of EWASs (including those in the meta-analyses) have been cross-sectional studies [11–15,17–20,22,24,25] and investigated differential methylation in combat-exposed populations and first responders [12,14–16,20,24,25]. PTSD symptoms often present differently in combat-exposed samples (increased hypervigilance and compulsive behaviour) compared to civilian samples [26,27]. PTSD symptom presentation, severity and recovery rates also differ between civilian trauma types [26,28,29]. Civilian EWASs have investigated a mixture of trauma types and none have investigated rape exclusively [30]. Civilian EWASs have also been predominantly conducted in mixed-gender [11,13,18,19,25,31], North American samples [11–13,17–20,25].

Ethnicity- and sex-specific characteristic may influence methylation profiles [32–34]. Women have a two-fold increased risk of developing PTSD compared to men [34]. Increased risk for PTSD in women may be X-chromosome linked, given that PTSD heritability is considerably higher among women compared to men [35,36]. Sex-specific expression of reproductive genes may also mediate the increased risk for PTSD in women e.g. estrogen levels have been associated with an altered hypothalamic-pituitary-adrenal (HPA) axis stress response in women [17,37,38] and differential methylation of estrogen response elements (EREs) in genes associated with HPA-axis functioning has been reported [17,39].

We sought to address the design shortcomings and demographic differences in prior EWASs by conducting a cross-sectional EWAS study, complemented by a candidate gene validation, replication and longitudinal investigation study of a demographically similar group of rape-exposed African black women in a low-income setting. Specific aims were to: (1) identify genome-wide differentially methylated CpG sites/regions associated with PTSD status at 3-months post-rape using an EWAS approach in a discovery set; (2) validating the findings resulting from the EWAS in selected genes at 3-months post-rape in a validation set; (3) replicate the findings in selected genes at 3-months post-rape in a replication set; (4) investigate if baseline methylation levels of selected genes predict PTSD status change over 6-months, in the combined set; (5) investigate if methylation changes in selected genes covary in relations to PTSD symptom scores over 6 months, in the combined set.

Table 1: *Summary of epigenome-wide association studies in PTSD*

Reference	Array and Tissue Type	Design and Sample Size	Setting and trauma type	Ethnicity	Gender & Mean Age	PTSD Measure	PTSD Associated Genes/Networks
Uddin et al., 2010[18]	HM27; Blood	Cross-sectional; 23 PTSD cases 77 trauma exposed controls	Civilians from the DNHS cohort; mixture of trauma types	79 African American, 14 Caucasian, 7 other ethnicities	40 Male (40%) 60 (60%) Female; 45.8 years	PCL-C	Functional annotation clustering of differentially methylated genes implicated genes associated with the immune system in the development of PTSD.
Smith et al., 2011[13]	HM27; Blood	Cross-sectional; 51 PTSD cases 53 trauma exposed controls	Civilians from the GTP cohort; mixture of trauma types	104 African American	64 Male (61.5%) 40 Female (38.5%); 42.7 years	CAPS	Genome-wide significant differences in methylation at CpG sites in the *APC5*, *TLR8*, *TPR*, *CLEC9A*, *ANXA2*.
Mehta et al., 2013[11]	450K; Blood	Cross-sectional 32 PTSD cases with CT 29 PTSD cases without CT	Civilian; mixture of trauma types	150 African American, 19 other ethnicities	18 Male (29.5%), 43 Female (70.5%); 41.6 years	PSS	Pathways affected by PTSD were related to apoptosis and cellular growth rate. Pathways uniquely affected in those with PTSD and CT were related to nervous system development and tolerance induction.
Chen, Kobayasji & Mellman, 2016[19]	450K; Blood	Cross-sectional; 12 PTSD cases 12 trauma exposed controls	Civilian; index traumas: 8 childhood physical or sexual abuse (33.3%); 3 sexual assault (12.5%); 9 violent crime (37.5%); 2 IPV (8.3%); 2 witnessed violent death (8.3%)	24 African American	13 Male (54.2%) 11 Female (45.8%); 22 years	CAPS	No genome-wide significant differences in methylation levels. Expression of genes associated with olfactory receptors, immune activation, GABAA receptor and vitamin D synthesis were upregulated in PTSD cases.
Hammamieh et al., 2017[12]	450K; Blood	Cross-sectional; 79 PTSD cases 80 combat trauma exposed controls	Combat exposed veterans previously deployed to Iraq or Afghanistan.	159 American ethnically matched participants (not otherwise specified)	159 Males (100%); 33.9 years	CAPS	Functional enrichment analysis of differentially methylated genes implicated genes related to nervous system

							development/functioning, somatic complications and endocrine signalling in the development of PTSD.
Kuan et al., 2017[20]	450K; Blood	Cross-sectional; 171 current PTSD cases 100 past PTSD cases 202 trauma exposed controls	Civilian responders to the September 11th World Trade Centre Disaster from the WTC cohort.	382 Caucasian American, 91 other ethnicities	473 Males (100%); 49.5 years	SCID	No genome-wide significant differences in methylation levels. Differential methylation at CpG sites in the *ZDHHC11*, *CSMD2*, *COL9A3*, *PDCD6IP*, *TBC1D24* and *FAM164A* genes were associated with current PTSD at a nominal level.
Mehta et al., 2017[14]	EPIC; Blood	Cross-sectional; 48 PTSD cases 48 combat exposed controls	Treatment seeking Vietnam veterans	96 Australian, not otherwise specified	96 Males (100%); 68.67 years	CAPS	Genome-wide significant differences in methylation at CpG sites in the *BRSK1*, *NGF*, *LCN8*, *DOCK2* genes and at an intergenic site (closest gene *LRRC3B*).
Kryzewska et al., 2018[24]	450K, Blood	Cross-sectional; 34 PTSD cases 39 trauma exposed controls	Police officers	73 Dutch	38 (52.1%) Males, 35 (47.9%) Females	CAPS	No genome-wide significant differences in methylation levels.
Maddox et al., 2018[17]	450K; Blood	Cross-sectional; 109 PTSD cases, 169 trauma exposed controls	Civilians from the GTP cohort; mixture of trauma types	278 predominately African American	278 (100%) Females	PSS	Genome-wide significant difference in methylation at one CpG sites in *HDAC4*.
Rutten et al., 2018[15]	450K; Blood	Discovery dataset: longitudinal; 32 high PTSD, high trauma 29 low PTSD, high trauma 32 low PTSD, low trauma Replication dataset: longitudinal; 35 with PTSD	Military soldiers pre- and post-deployment (minimum of 4 months) to Afghanistan from the PRISMO cohort. Marines pre- and post-deployment to Iraq or	93 Dutch Caucasian soldiers and 98 North American marines	93 Males (100%); 27.5 years and 98 Males (100%); 22 years	SRIP or CAPS	Longitudinal changes in PTSD symptoms were associated with differential methylation at CpG sites in the *DUSP22*, *NINJ2*, *HOOK2*, *SDK1*, *MYT1L*, *PAX8*, *COL1A2* and *HIST1H2APS2* genes in the PRISMO cohort. The finding related to *HIST1H2APS2* was replicated in the MRS cohort.

		63 controls with combat exposure	Afghanistan from the MRS cohort.				
Uddin et al., 2018[21]	450K, Blood	Cross-sectional, meta-analysis 198 with PTSD 347 trauma exposed controls	Civilians from the DNHS, GTP and WTC cohorts; mixture of trauma types.	343 African American, 164 Caucasian American, 38 other ethnicities	294 Males (54%), 251 Females (46%), 46.6 years	PCL-C CAPS SCID	Genome-wide significant differences in methylation of CpG sites in the *NRG1* and *HGS* genes.
Logue et al., 2020[25]	EPIC, Blood	Cross-sectional 378 PTSD cases 135 trauma exposed controls	War veterans exposed to combat trauma in Iraq and/or Afghanistan form the TRACTS cohorts and veterans recruited from TBI-VA-Boston.	513 American veterans (not otherwise specified)	467 (91%) Males, 46 (9%) Females, 32.7 years	CAPS	Genome-wide significant difference in methylation of a CpG site in the *G0S2* gene.
Snijders et al., 2020[16]	450K; Blood	Longitudinal 123 PTSD cases 143 war exposed controls	Military (marine and army) combat exposed personnel from the MRS, STARRS and PRISMO cohorts. Deployed to Iraq or Afghanistan for 4 to 7 months.	126 predominately Caucasian American marines, 78 Caucasian American army soldiers, 62 Dutch army soldiers	266 (100%) Males; 24.5 years	CAPS, PCL/CID I-SC and SRIP	Genome-wide significant differences in methylation of CpG sites in the *SPRY4*, *SDK1*, *CTRC*, *CDH15*, *MAD1L1*, *HEXDC* genes.
Smith et al., 2019[22]	450K, Blood	Cross-sectional, meta-analysis 878 PTSD cases 1018 trauma exposed controls	Three civilian samples and seven combat samples all exposed to trauma including combat and various civilian traumas. DNHS, GTP, WTC, STARRS, MRS, INTRusST, PRISMO, VA-M-EA, VA-M-AA and VA-NCPTSD cohorts	986 Caucasian American, 62 Dutch, 777 African American, 57 Hispanic, 76 other ethnicities.	1303 (68.7%) Males, 593 (31.3%) Females, 35.8 years	PCL-C, DSM-IV, CAPS, MINI, SCID, CIDI-SC, SRIP	Genome-wide significant differences in methylation of CpG sites in the *AHRR*, *RNF6*, *MIR3170*, *ATP9A*, *AC011899.9*, *FLJ46321* and *LINC00599* genes.

Abbreviations:

HumanMethylation27 BeadChip (HM27); posttraumatic stress disorder (PTSD); Detroit Neighborhood Health Study (DNHS); PTSD Checklist – Civilian Version (PCL-C); Grady Trauma Project (GTP); Clinician-Administered PTSD Scale (CAPS); acid phosphatase 5, tartrate resistant (APC5); toll-like receptor 8 *(TLR8)*; translocated promoter region *(TPR)*; C-type lectin domain family 9, member A *(CLEC9A)*; annexin A2 *(ANXA2)*; HumanMethylation 450K BeadChip (450K); childhood trauma (CT); PTSD Symptom Scale (PSS); intimate partner violence (IPV); gamma-aminobutyris acid A (GABAA); World Trade Centre 9/11 responders study (WTC); Structured Clinical Interview for DSM Disorders (SCID); zinc finger DHHC-type containing 11 *(ZDHHC11)*; CUB and sushi domain-containing protein *(2CSMD2)*; collagen type IX alpha 3 chai *(COL9A3)*; programmed cell death 6 interacting protein *(PDCD6IP)*; TBC1 domain family member 24 *(TBC1D24)*; family with sequence similarity 164, member A *(FAM164A)*; MethylationEPIC BeadChip (EPIC); brain-specific serine/threonine-protein kinase 1 *(BRSK1)*; nerve growth factor *(NGF)*; lipocalin 8 *(LCN8)*; dedicator of cytokinesis 2 *(DOCK2)*; leucine rich repeat containing 3B *(LRRC3B)*; histone deacetylase 4 *(HDAC4)*; Prospective Research in Stress-related Military Operations (PRISMO); Marine Resiliency Study (MRS); Self-Rating Inventory for PTSD (SRIP); dual specificity phosphatase 22 *(DUSP22)*; ninjurin 2 *(NINJ2)*; hook microtubule tethering protein 2 *(HOOK2)*; sidekick cell adhesion molecule 1 *(SDK1)*; myelin transcription factor 1 like *(MYT1L)*; paired box 8 *(PAX8)*; collagen type I alpha 2 chain *(COL1A2)*; H2A histone family, member T, pseudogene *(HIST1H2APS2)*; neuregulin 1 *(NRG1);* hepatocyte growth factor-regulated tyrosine kinase substrate *(HGS);* Translational Research Centre for TBI and Stress Disorders (TRACTS); Department of Veterans Affairs Rehabilitation Research and Development (VA-RR&D); Traumatic Brain Injury Centre of Excellence – Veteran Affairs Boston Healthcare System (TBI-VA-Boston); G0/G1 switch 2 *(G0S2)*; Study to Assess Risk and Resiliency in Servicemembers (STARRS); Composite International Diagnostic Interview – Screening Scales (CIDI-SC); sprouty RTK signalling antagonist 4 *(SPRY4)*; sidekick cell adhesion molecule 1 *(SDK1)*; chymotrypsin C *(CTRC)*; cadherin 15 *(CDH15)*; mitotic arrest deficient 1 like 1 *(MAD1L1)*; hexosaminidase glycosyl hydrolase family 20 catalytic domain containing *(HEXDC)*; Injury and Traumatic Stress Study (INTRusST); Mid-Atlantic Mental Illness Research Education and Clinical Center PTSD Study European American cohort (VA-M-EA) and African American cohort (VA-M-AA); Boston Veterans Affairs National Center for PTSD (VA-NCPTSD); Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV); Mini-International Neuropsychiatric Interview (MINI); human aryl hydrocarbon receptor repressor *(AHRR)*; ring finger protein 6 *(RNF6)*; microRNA 3170 *(MIR3170)*; ATPase phospholipid transporting 9A *(ATP9A)*; family with sequence similarity 75, member D1 *(FLJ46321)*; long intergenic non-protein coding RNA 599 *(LINC00599)*.

METHODS

Participant recruitment and setting

Samples and data were analysed from a discovery set (n = 48), a validation set (n = 48), a replication set (n = 49) and the combined validation and replication set (n = 96). The Participants were recruited through the Rape Impact Cohort Evaluation (RICE) study conducted in South Africa (n = 852). A detailed description of the methods of the RICE study has been published elsewhere [40]. In short, female survivors of rape were recruited from rape clinics. Interested participants were invited to the study site to enrol in the study following informed consent procedures. Recruitment was restricted to female participants between 18 and 40 years who reported a rape in the preceding 20 days of the baseline visit. In the current study, we excluded women who: (1) were pregnant or lactating during the course of the study; (2) met criteria for PTSD at the baseline visit, indicative of PTSD due to a past traumatic event other than the rape; and (3) had HIV-seroconverted.

Ethical approval for RICE was obtained from the Human Research Ethics Committee at the South African Medical Research Council (SAMRC; EC019-10/2013) and approval to conduct the sub-study was obtained from the Health Research Ethics Committee at Stellenbosch University (S16/08/146).

Clinical measures

At the baseline visit, a research assistant supervised by a registered trauma counsellor or registered nurse assessed for PTSD (in relation to prior criterion A traumas other than the rape) on the Mini International Neuropsychiatric Interview (MINI) version 7.0.0 [41]. An HIV rapid test, pregnancy test, blood collection for DNA analysis and assessment of body mass index (BMI) were undertaken by a nurse at all timepoints (baseline, 3-months and 6-months post-rape).

A research assistant administered a demographic questionnaire, a modified version of the Childhood Trauma Questionnaire - Short Form (CTQ-SF) [42] and a modified version of the Life Events Checklist (LEC) [43,44] at baseline. The Davidson Trauma Scale (DTS) [45], the Alcohol Use Disorders Identification Test, alcohol consumption subscale (AUDIT-C) [46] and the Center for Epidemiologic Studies Depression Scale (CES-D) [47] was administered at all timepoints. The DTS was used to measure PTSD symptoms with a cut-off score of forty or more considered indicative of PTSD [45]. This cut-off was used to group participants into PTSD cases and controls at 3-months post-rape (see supplementary material for more details) [45]. All

assessments were completed face-to-face and responses were recorded and electronically captured in real-time on a secure server. Item-level missing values were imputed using a multiple imputation model while maintaining a multivariate normal distribution.

Demographic and clinical characteristics of the sample

The baseline demographic and clinical characteristics of the sample were investigated using descriptive statistics. Differences in baseline demographic and clinical characteristics between the discovery/validation set and the replication set were investigated using non-parametric tests, since most of the variables did not conform to a normal distribution. Mann-Whitney U tests were used to compare differences between the groups for the continuous variables, i.e., age, body mass index (BMI), childhood trauma score, number of childhood traumas endorsed, number of lifetime traumas endorsed, alcohol use and depression symptom scores. Chi-square statistics were used to compare differences between the groups on several categorical variables (completed secondary education, relationship status, smoking status, HIV status, medication use, childhood neglect, witnessed domestic violence in childhood home, childhood emotional abuse, childhood physical abuse, childhood sexual abuse, imprisonment, civil unrest or war, serious injury, being close to death, murder of a family member or friend, unnatural death of a family member or friend, murder of a stranger, robbed at gun/knife point, kidnapped, hazardous alcohol use and depression status).

The same variables and methods used to investigate baseline demographic and clinical differences between the discovery/validation and replication sets were used to investigate differences between those with and without PTSD at 3-months post-rape.

Cross-sectional analyses (3 months post-rape)

Discovery set

Forty-eight participants, 24 with PTSD and 24 without PTSD at 3-months post-rape, were included in the discovery set. We implemented a cross-sectional, case-control EWAS design to identify genome-wide differentially methylated positions (DMPs) and differentially methylated regions (DMRs) between those with and without PTSD. Consecutive cases of PTSD at 3-months post-rape were identified until the target number was reached. Controls were perfectly matched to cases based on HIV status and as closely as possible (in descending hierarchical order of importance) on age, childhood trauma scores, lifetime trauma exposure, BMI, smoking, education and income. DNA was extracted from peripheral blood samples and

assayed using the Human MethylationEPIC BeadChip array (Illumina, California, United States).

Raw probe intensity data (iDAT) files produced by Illumina GenomeStudio were decompressed and parsed into text format using the *meffil* R package [49] in R statistics version 3.6.2 [50]. All EWAS analyses, including quality control measures and beta normalisation, were completed using the *meffil* R package [49].

No samples were excluded from the analysis, given that all samples passed the quality control checks (see Supplementary Material and Supplementary Figures 1, 2, 4 and 5). Probes not passing the quality control checks (n = 29936) were excluded from the downstream analyses (see Supplementary Material and Supplementary Figure 2 and 5). Previously identified cross-reactive probes for 43254 CpG sites were also excluded [51]. Probes targeting CpG sites on the X chromosome were retained since all participants included in the study were female.

The percentage of methylated alleles for each CpG site in each sample was calculated as $\beta = M / (M + U + 100)$ where M and U symbolises raw probe fluorescent intensities for methylated and unmethylated signals, respectively [52]. Technical bias and batch effects were corrected for using functional normalisation (Supplementary Material, Supplementary Figure 7-11, Supplementary Tables 1 and 2) [53]. Any residual effects were handled by including surrogate variables as covariates in the EWAS models. These were estimated following functional normalisation using surrogate variable analysis (SVA) [54]. Cell type composition was estimated by applying the Houseman algorithm to the normalized DNA methylation profiles and a publicly available blood cell type reference dataset (Gene Expression Omnibus accession number GSE35069) [55]. Cell type composition was included in the final EWAS models (Supplementary Figure 12). Epigenome-wide associations were investigated using logistic regression models to identify DMPs associated with PTSD status. Benjamini-Hochberg correction was applied to correct for multiple testing with an adjusted p-value < .01 indicating genome-wide significance [56].

The *dmrff* R package was applied to EWAS summary statistics to identify DMRs [57]. DMRs were defined as a region covering two or more CpG sites with less than 100 bp between consecutive sites showing the same direction of effect with an uncorrected p-value < .05 (see supplementary material for more details) [57]. A DMR was considered significant on an epigenome-wide level if a Bonferroni-adjusted $p < 0.05$ was observed. Coordinates resulting from the DMP and DMR analyses were annotated using the MethylationEPIC_v-1-0_B4 manifest [52]. Co-variation in methylation levels between blood and brain tissue was explored

using the online Blood Brain DNA Methylation Comparison Tool [58]. Prior findings reporting a link between any exposure or phenotype and the CpG sites identified from the EWAS were identified using the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) catalogue of epigenome-wide association studies[59] and the China National Center for Bioinformation National Genomics Data Center epigenome-wide association studies (EWAS) atlas[60]. All genomic coordinates reported in this study is in reference to the Hg19/GRCh37 human genome assembly (see Supplementary Material and Supplementary Figures 13 for more details).

Validation set

A candidate gene approach was used to validate the findings of the EWAS. The same sample included in the discovery set were investigated in the validation set. Samples were assayed using EpiTYPER Sequenom MassARRAY technology (Agena Bioscience, California, United States). DNA methylation was investigated at CpG sites in two selected regions at 3-months post-rape. Brain-specific serine/threonine-protein kinase 2 (*BRSK2)* and adenylate cyclase activating polypeptide 1 *(ADCYAP1)* were selected as candidate genes since they were the only genes that reached statistical significance as DMPs in the EWAS, prior to correction for multiple testing, and reached statistical significance at a genome-wide level as DMRs. *BRSK1,* a paralog of *BRSK2* was identified as a DMP in a prior PTSD EWAS study [14]. *ADCYAP1* receptor 1 *(ADCYAP1R1)* has been linked to the development of PTSD in several prior studies [14,61–63].

DNA methylation percentages were exported using the EpiTYPER Analyzer software (see Supplementary Material for more details). The validation analyses were completed using IBM SPSS Statistics 27.0. Logistic regression models were used to determine if differential methylation of *BRSK2* and *ADCYAP1* at 3-months post-rape was associated with PTSD status at 3-months post-rape.

The relationship between baseline confounding variables, PTSD status at 3-months post-rape, *BRSK2* methylation at 3-months post-rape and *ADCYAP1* methylation at 3-months post-rape were investigated using Mann-Whitney U tests, Chi-square and Spearman's correlations. Potential confounders included the continuous variables age, BMI, childhood trauma score, number of lifetime traumas endorsed, alcohol use and depression, and the categorical variables HIV status, smoking and medication use. Confounding variables significantly associated with PTSD or *BRSK2/ADCYAP1* methylation were entered in the logistic regression models as covariates, in a stepwise manner.

Replication set

Forty-nine additional, consecutively selected, participants from the RICE study were investigated in the replication set. Participants included in this replication set were not matched on PTSD status or potential methylation covariates. Samples were assayed using EpiTYPER Sequenom MassARRAY technology (Agena Bioscience, California, United States).

Logistic regression models, including potential confounding variables, were used to determine if differential methylation of *BRSK2* and *ADCYAP1* at 3-months post-rape was associated with PTSD status at 3-months post-rape in the replication set, following the same procedure applied in the validation set.

Comparison of previous findings from candidate gene studies and EWASs

Candidate gene studies and EWASs investigating the relationship between methylation and PTSD were identified from published literature. For EWASs, the Illumina CpG identification number for significant findings was manually recorded and cross-checked against the findings of the current EWAS. For candidate gene studies, the genomic coordinates of the sites were identified from the publications and converted to H19/GRCh37 positions using the BLAT function of the UCSC genome browser (if not already indicated as H19/GrCh37 positions). The genomic locations were manually recorded and cross-checked with the MethylationEPIC_v-1-0_B4 manifest to determine if the sites were included on the MethylationEPIC array. Significant CpG sites resulting from the current EWAS and corresponding to prior findings are reported in the results.

Agreement between the MethylationEPIC array and EpiTYPER

Spearman's correlation coefficients were used to investigate the level of agreement between methylation levels resulting from the MethylaytionEPIC array at 3-months post-rape and methylation levels resulting from EpiTYPER at 3-months post-rape.

Longitudinal investigation (baseline, 3-months and 6-months post-rape)

Combined set

The validation and replication sets were combined and methylation data from the baseline and 6-month post-rape samples were added to the dataset, for the same combined group. The group consisted of 96 participants with methylation data at all timepoints (baseline, 3-months and 6-months). The samples were assayed using EpiTYPER Sequenom MassARRAY technology (Agena Bioscience, California, United States). We investigated the same *BRSK2* and

ADCYAP1 CpG sites investigated in the validation and replication sets but followed a longitudinal cohort design with PTSD symptom scores as outcome, instead of a cross-sectional case-control design with PTSD status at 3-months as outcome.

The PTSD scores at each timepoint were compared between the discovery/validation set and the replication set using Mann-Whitney U tests. The relationship between PTSD, *BRSK2* methylation, *ADCYAP1* methylation (at all timepoints) and potential baseline confounders (age, BMI, childhood trauma, lifetime traumas, alcohol use, depression, HIV status, smoking and medication use) were investigated using Mann-Whitney U tests, Chi-square and Spearman's correlations.

Baseline *ADCYAP1* and *BRSK2* methylation levels were investigated as predictors of change in PTSD symptom scores over six months, in the first set of mixed regression models. In the second set of mixed regression models, we investigated change in *BRSK2* and *ADCYAP1* methylation levels over six months in relation to change in PTSD symptom scores over six months. Confounding variables significantly associated with PTSD or *BRSK2/ADCYAP1* methylation at any timepoint were entered in the mixed regression models as covariates, in a stepwise manner.

RESULTS

Baseline demographic and clinical characteristics of the sample

Table 2 presents the baseline demographic and clinical characteristics of the discovery/validation and replication sets. The sets were similar in demographic and clinical characteristics. The only variable that differed between the sets was the prevalence of lifetime exposure to the murder of a family member or friend, which was more frequently endorsed in the discovery/validation set compared to the replication set (25.5% vs. 8.2%, respectively; χ^2 = 5.2, p = .022).

Table 2: *Baseline demographic and clinical characteristics of the discovery/validation and replication sets*

	Discovery/validation set (n=47)		Replication set (n=49)		Comparison of Discovery/validation set to replication set		
	n (%)	*M (SD)*	n (%)	*M (SD)*	χ^2	*z*	*p*
Age[1]	47(100)	25.9(5.4)	49(100)	24.6(5.5)		-1.3	.178
Secondary education completed[2]	32(68.1)		25(51)		2.9		.089
Employed[2]	13(27.7)		9(18.4)		1.2		.279
In a relationship/married[2]	38(80.9)		38(77.6)		0.2		.691
BMI[1]	47(100)	26.0(6.5)	49(100)	25.8(5.7)		-0.1	.956
Smoker[2]	5(10.6)		7(14.3)		0.3		.589
HIV positive[2]	27(57.4)		19(38.8)		3.4		.067
On ARVs[2]	12(25.5)		14(28.6)		0.1		.738
On medications for STI[2]	2(4.3)		2(4.1)		0.0		.966
Other medication use[2,3]	1(2.1)		2(4.1)		0.3		.582
Childhood trauma score[1]	47(100)	17.2(4.1)	49(100)	16.2(2.5)		-0.8	.410
Neglect[2]	23(48.9)		18(36.7)		1.5		.227
Domestic violence[2]	10(21.3)		8(16.3)		0.4		.534
Emotional abuse[2]	12(25.5)		11(22.4)		0.1		.724
Physical abuse[2]	18(38.3)		19(38.8)		0.0		.962
Sexual abuse[2]	10(21.3)		11(22.4)		0.0		.890
Number of childhood traumas[1]	47(100)	1.6(1.6)	49(100)	1.4(1.5)		-0.6	.530
Number of lifetime traumas[1,4]	47(100)	1.6(1.5)	49(100)	1.1(1.2)		-1.7	.092
Imprisonment[2]	2(4.3)		1(2.0)		0.4		.533
Civil unrest or war[2]	3(6.4)		1(2.0)		1.1		.287
Serious injury[2]	8(17.0)		3(6.1)		2.8		.094
Being close to death[2]	13(27.7)		14(28.6)		0.0		.921
Murder of family/friend[2]	12(25.5)		4(8.2)		5.2		.022*
Unnatural death family/friend[2]	9(19.1)		5(10.2)		1.5		.214
Murder of stranger[2]	10(21.3)		5(10.2)		2.2		.135
Robbed gun/knife used[2]	17(36.2)		18(36.7)		0.0		.954
Kidnapped[2]	3(6.4)		4(8.2)		0.1		.737
PTSD symptom score[1]	47(100)	67.1(21.7)	49(100)	65.7(18.6)		-0.8	.431
Alcohol use severity[1]	47(100)	1.4(2.2)	49(100)	1.9(2.5)		-1.2	.242
Hazardous alcohol use[2]	12(25.5)		15(30.6)		0.3		.580
Depression symptom score[1]	47(100)	32.4(13.9)	49(100)	31.7(12.1)		-0.2	.854
Depression status[2]	41(87.2)		45(91.8)		0.5		.461

[1]Continous variables; [2]categorical variables; [3]medication prescribed for chronic sinusitis (n=1) and hypertension (n=2); [4]lifetime traumas refer to directly experiencing the trauma; *p < .05
Abbreviations: Posttraumatic stress disorder (PTSD); mean (M); standard deviation (SD); body mass index (BMI); antiretrovirals (ARV); sexually transmitted infection (STI).

Comparison of baseline demographic and clinical characteristics between the PTSD groups at 3-months post-rape

Table 3 presents the findings of the PTSD status group comparisons (at 3-months post-rape) in the discovery/validation set and the replication set, consecutively. Participants with and without PTSD had similar baseline demographic and clinical characteristics in the discovery/validation and replication sets. However, in the discovery/validation set, those with PTSD were more likely to endorse being robbed with a gun or knife compared to those without PTSD (50% and 21.7%, respectively; $z = 4.1$, $p = .044$). In the replication set, those with PTSD endorsed less lifetime traumas ($M = 0.5$, $SD = 0.7$) compared to those without PTSD ($M = 1.4$, $SD = 1.3$, $z = -2.5$, $p = .014$).

Table 3: *Baseline demographic and clinical characteristics of rape-exposed participants with and without PTSD in the discovery/validation and replication sets*

	Discovery/validation set (n = 47)							Replication set (n = 49)						
	With PTSD at 3-months (n = 24)		Without PTSD at 3-months (n = 23)		Group differences			With PTSD at 3-months (n = 15)		Without PTSD at 3-months (n = 34)		Group differences		
	n (%)	*M(SD)*	n (%)	*M(SD)*	χ^2	*z*	*p*	n (%)	*M(SD)*	n (%)	*M(SD)*	χ^2	*z*	*p*
Age[1]	24(100)	25.1(5.3)	23(100)	26.7(5.5)		-1.0	.296	15(100)	24.7(4.7)	34(100)	24.5(5.9)		-0.5	.616
Secondary education completed[2]	16(66.7)		16(69.6)		0.1		.831	10(66.7)		15(44.1)		2.1		.146
Employed[2]	4(16.7)		9(39.1)		3.0		.085	1(6.7)		8(23.5)		2.0		.160
In a relationship/married[2]	19(79.2)		19(82.6)		0.1		.764	11(77.3)		27(79.4)		0.2		.638
BMI[1]	24(100)	24.8(5.4)	23(100)	27.2(7.4)		-1.1	.268	15(100)	25.3(4.8)	34(100)	26.0(6.1)		-0.3	.745
Smoker[2]	3(12.5)		2(8.6)		0.2		.672	2(13.3)		5(14.7)		0.0		.899
HIV positive[2]	14(58.3)		13(56.5)		0.0		.900	7(46.7)		12(35.3)		0.6		.451
On ARVs[2]	6(25.0)		6(26.1)		0.0		.932	4(26.7)		10(29.4)		0.0		.845
On medications for STI[2]	1(4.2)		1(4.3)		0.0		.975	0(0.0)		2(5.9)		0.9		.338
Other medication use[2,3]	0(0.0)		1(4.3)		1.1		.302	0(0.0)		2(5.9)		0.9		.338
Childhood trauma score[1]	24(100)	18.2(4.6)	23(100)	16.2(3.3)		-1.7	.098	15(100)	15.7(2.5)	34(100)	16.4(2.6)		-1.0	.299
Neglect[2]	13(54.2)		10(43.5)		0.5		.464	5(33.3)		13(38.2)		0.1		.743
Domestic violence[2]	7(29.2)		3(13.0)		1.8		.177	3(20.0)		5(14.7)		0.2		.644
Emotional abuse[2]	9(37.5)		3(13.0)		3.7		.055	3(20.0)		8(23.5)		0.1		.785
Physical abuse[2]	10(41.7)		8(34.8)		0.2		.627	5(33.3)		14(41.2)		0.3		.604
Sexual abuse[2]	7(29.2)		3(13.0)		1.8		.177	2(13.3)		9(26.5)		1.0		.310
Number of childhood traumas[1]	24(100)	1.9(1.7)	23(100)	1.2(1.3)		-1.6	.120	15(100)	1.2(1.7)	34(100)	1.4(1.5)		-0.8	.430
Number of lifetime traumas[1,4]	24(100)	2.0(1.6)	23(100)	1.2(1.2)		-1.9	.063	15(100)	0.5(0.7)	34(100)	1.4(1.3)		-2.5	.014
Imprisonment[2]	2(8.3)		0(0.0)		2.0		.157	0(0.0)		1(2.9)		0.5		.502
Civil unrest or war[2]	2(8.3)		1(4.3)		0.3		.576	0(0.0)		1(2.9)		0.5		.502
Serious injury[2]	6(25.0)		2(8.6)		2.2		.137	1(6.7)		2(5.9)		0.0		.916
Being close to death[2]	7(29.2)		6(26.1)		0.1		.813	3(20.0)		11(32.4)		0.8		.378
Murder of family/friend[2]	5(20.8)		7(30.4)		0.6		.450	0(0.0)		4(11.8)		1.9		.166
Unnatural death family/friend[2]	5(20.8)		4(17.4)		0.1		.764	0(0.0)		5(14.7)		2.5		.117
Murder of stranger[2]	7(29.2)		3(13.0)			1.8	.177	0(0.0)		5(14.7)		2.5		.117

Robbed gun/knife used[2]	12(50.0)		5(21.7)		4.1	.044	3(20.0)		15(44.1)		2.6		.107
Kidnapped[2]	3(12.5)		0(0.0)		3.1	.080	1(6.7)		3(8.8)		0.1		.799
PTSD symptom score[1]	24(100)	75.7(17.9)	23(100)	58.1(22.0)	-2.9	.004	15(100)	63.4(20.4)	34(100)	66.7(18.0)	-0.1		.914
Alcohol use severity[1]	24(100)	1.7(2.4)	23(100)	1.2(1.9)	-0.9	.394	15(100)	1.6(2.6)	34(100)	2.1(2.5)		-1.0	.299
Hazardous alcohol use[2]	7(29.2)		5(21.7)		0.3	.559	4(26.7)		11(32.4)		0.2		.691
Depression symptom score[1]	24(100)	35.1(12.9)	23(100)	29.5(14.7)	-1.4	.173	15(100)	28.4(13.5)	34(100)	33.1(11.4)		-1.4	.149
Depression status[2]	22(91.7)		19(82.6)		0.9	.352	13(86.7)		32(94.1)		0.8		.380

[1]Continous variables; [2]categorical variables; [3]medication prescribed for chronic sinusitis (n=1) and hypertension (n=2); [4]lifetime traumas refer to directly experiencing the trauma.

Abbreviations: Posttraumatic stress disorder (PTSD); mean (M); standard deviation (SD); body mass index (BMI); antiretrovirals (ARV); sexually transmitted infection (STI).

Discovery set: genome-wide differentially methylated genes associated with PTSD status at 3-months post-rape

Table 4 presents the findings of the top twenty DMPs that were associated with PTSD before correction for multiple comparisons ($p < .05$; see supplementary Figure 14 & 15 for more details). Only one DMP, cg01700569, remained significant after correcting for multiple testing (FDR adjusted $p < .01$). This intergenic site (cg01700569) is located 24 694 bases downstream of solute carrier family 16 member 9 (*SLC16A9*). Thirty-four DMRs were identified from the regional analysis after Bonferroni correction for multiple testing (adjusted $p < .05$; see supplementary Table 3 for details). Four regions were not attributed to a gene (chr1:153762201-153762244, chr17:72595585-72595587, chr1:153762359-153762434, chr1:153762201-153762244). The remaining regions were associated with leucine-rich repeat-containing protein 34 (*LRRC34*; three regions), phospholipase D family member 6 (*PLD6*), solute carrier family 38 member 11 (*SLC38A11*), coiled-coil and C2 domain-containing protein 2A (*CC2D2A*), endothelial cell specific molecule 1 (*ESM1*; two regions), sorbin and SH3 domain-containing protein 2 (*SORBS2*), ankyrin repeat domain-containing protein 33 (*ANKRD33*), collagen type XVIII alpha 1 chain (*COL18A1*), *BRSK2*, *AC004895.4*, spondin 1 (*SPON1*), *ADCYAP1*, inositol polyphosphate-5-phosphatase A (*INPP5A*), chromosome 21 open reading frame 62 *(C21orf62)*, *RP11-1085N6.3*, thrombospondin type laminin G domain and EAR repeats antisense RNA 2 *(TSPEAR-AS2)*, microRNA 125b-1 *(MIR125B1)*, chromosome 5 open reading frame 66 antisense RNA *(C5orf66AS1)*, fascin actin-bundling protein 2 *(FSCN2)*, forkhead box J3 *(FOXJ3)*, solute carrier family 39 member 13 *(SLC39A13)*, tubulin polymerization promoting protein *(TPPP)*, long intergenic non protein coding RNA 1529 *(LINC01529)*, chromosome 5 open reading frame 63 *(C5orf63)*, hepatocellular carcinoma-associated gene 2 *(HCCA2)*, oxytocin/neurophysin I prepropeptide *(OXT)* and zinc finger protein 595 *(ZNF595)* genes.

Table 4: *Genome-wide differentially methylated positions (DMPs) and regions (DMRs) associated with PTSD*

Gene Name[1]	Position[2]	Probe	Relation to Island[3]	Location in Gene[3,4]	*β*	SE	*t/z*	*p*	Adj. *p*	Other exposures/ phenotypes associated with the CpG site[5]
Differentially methylated positions (DMPs)										
NA, *SLC16A9*[6]	Chr10:61385771	cg01700569	OpenSea	NSF	0.031	0.004	7.119	6.187e-08	0.049233	None
NA, *IRF4*[6]	Chr6:429318	cg06868375	OpenSea	NSF	-0.009	0.002	-5.846	2.067e-06	0.777284	None
FEZ1	Chr11:125365803	cg06309855	Island	5'UTR	0.022	0.004	5.727	2.930e-06	0.777284	Gestational age
CGLC	Chr6:53371893	cg05277169	OpenSea	Body	0.013	0.002	5.468	6.043e-06	0.999998	None
BEGAIN	Chr14:101036470	cg05730092	S_Shore	TSS1500	0.032	0.006	5.401	7.352e-06	0.999998	Gestational age
CRTC3-AS1	Chr15:91193536	cg16207883	OpenSea	Body	0.021	0.004	5.233	1.183e-05	0.999998	None
LINC01006	Chr7:156267149	cg01020356	OpenSea	Body	0.025	0.005	5.224	1.216e-05	0.999998	Ethnicity; osteonecrosis of the femoral head
EPB41L1	Chr20:34818105	cg20355257	OpenSea	3'UTR	0.017	0.003	5.204	1.286e-05	0.999998	Down syndrome
ADCYAP1	Chr18:905177	cg22388954	Island	5'UTR; TSS200	-0.025	0.005	-5.181	1.371e-05	0.999998	B acute lymphoblastic leukemia
BRSK2	Chr11:1431833	cg09450823	Island	Body	0.036	0.007	5.115	1.653e-05	0.999998	None
NA, *MIR4290*[6]	Chr9:92782655	cg04299914	OpenSea	NSF	-0.049	0.010	-5.091	1.769e-05	0.999998	None
USP49	Chr6:41790313	cg11943190	OpenSea	5'UTR	-0.010	0.002	-5.050	1.970e-05	0.999998	Rheumatoid arthritis; exercise
DOK5	Chr20:53127455	cg01998039	OpenSea	5'UTR; Body	0.040	0.008	5.049	1.994e-05	0.999998	Down syndrome; aging
MCEE	Chr2:71338735	cg08848660	OpenSea	Body	-0.011	0.002	-5.012	2.205e-05	0.999998	None

CTNNA3	Chr10:68940214	cg23307744	OpenSea	Body	-0.012	0.002	-5.012	2.208e-05	0.999998	None
NA, *AC004854.2*[6]	Chr7:44965393	cg01855601	OpenSea	NSF	0.010	0.002	4.975	2.436e-05	0.999998	None
PARD3	Chr10:35016204	cg18026072	OpenSea	Body	-0.009	0.002	-4.961	2.534e-05	0.999998	None
NA, *BRSK2*[6]	Chr11:1401914	cg22820726	N_Shelf	NSF	-0.063	0.013	-4.963	2.544e-05	0.999998	None
TMEM52B	Chr12:10322100	cg05617790	OpenSea	Body; TSS1500	0.015	0.003	4.953	2.619e-05	0.999998	None
TRMO	Chr9:100662939	cg13109115	OpenSea	NSF	-0.103	0.021	-4.915	2.915e-05	0.999998	None

[1] Identified using the GENECODE database; [2] identified using the Human Genome 19 (HG19) build from the Genome Reference Consortium; [3] identified using the University of California Santa Cruz (UCSC) Genomic Institute/Genome Browser; [4] multiple listings indicate splice variants; [5] identified using the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) catalog of epigenome-wide association studies[59] and the China National Center for Bioinformation National Genomics Data Center epigenome-wide association studies (EWAS) atlas[60]; [6] CpG sites located in a region not attributed to a gene, the gene closest to the CpG site is provided; [7] The site is associated with a promoter region - identified using the Methylation Consortium project.

Abbreviations: Standard error (SE); adjusted (Adj); not applicable (NA); solute carrier family 16 member 9 *(SLC16A9)*; not specified (NSF); interferon regulatory factor 4 *(IRF4)*; fasciculation and elogation protein zeta 1 *(FEZ1)*; 5' untranslated region (5'UTR); glutamate-cysteine ligase catalytic subunit (*GCLC);* brain-enriched guanylate kinase-associated protein *(BEGAIN)*; transcription start site 1500 (TSS1500); South shore (S_Shore); CRTC3 antisense RNA 1 (*CRTC3-AS1)*; long intergenic non-protein coding RNA 1006 *(LINC01006);* erythrocyte membrane protein band 4.1 Like 1 *(EPB41L1)*; 3' untranslated region (3'UTR); adenylate cyclase activating polypeptide 1 *(ADCYAP1)*; transcription start site 200 (TSS200); brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; microRNA 4290 *(MIR4290)*; ubiquitin specific peptidase 49 *(USP49)*; docking protein 5 *(DOK5)*; methylmalonyl-CoA epimerase *(MCEE)*; catenin alpha 3 *(CTNNA3)*; novel transcript antisense to purine rich element binding protein B *(AC004854.2)*; PAR-3 family cell polarity regulator (*PARD3)*; North shelf (N_Shelf); transmembrane protein 52B *(TMEM52B)*; TRNA methyltransferase O (*TRMO).*

Validation and replication set: differential methylation of *BRSK2* in relation to PTSD status at 3-months post-rape

The *BRSK2* region (chr11:1463541-1463670; adjusted $p < .05$) identified from the EWAS included five CpG sites (CpG1 - cg12186219, CpG2 - cg14064268, CpG3 - cg10590925, CpG4 - cg17429870, CpG5 - cg18651858) that showed decreased methylation in participants with PTSD (see Figure 1). Based on prior findings, DNA methylation of these CpG sites in blood was highly correlated with DNA methylation in the prefrontal cortex, superior temporal gyrus and the cerebellum (see Supplementary Figures 16a-16d) [58] Three of the five CpG sites (CpG3, CpG4 and CpG5) were investigated in the validation and replication set. We could not investigate CpG1 or CpG2, as the mass of CpG1 was too low to be measured by the EpiTYPER mass spectrometer, and CpG2 contained a silent peak that overlapped with the non-methylated peak for this site (see supplementary Table 4 for the genomic coordinates and sequence for CpG3, CpG4 and CpG5).

Baseline age, HIV status, BMI, smoking status, childhood trauma score, lifetime trauma, alcohol use, depression and medication use were not associated with *BRSK2* methylation at 3-months post-rape in either the validation or replication sets. PTSD status at 3-months post-rape was associated with lifetime trauma ($z = -2.47$, $p = .014$) in the replication set only (see supplementary Table 5 & 6).

In the validation set, methylation levels of *BRSK2* CpG3 ($\beta = -0.04$, $p = .050$, OR 0.96) and CpG4 ($\beta = -0.04$, $p = .052$, OR 0.96) at 3-months post-rape were not significantly associated with PTSD status at 3-months post-rape. Decreased methylation of *BRSK2* CpG5 ($\beta = -0.04$, $p = .048$, OR 0.96) at 3-months post-rape was significantly associated with a PTSD status at 3-months post-rape, but the association was no longer significant when lifetime trauma was added as a covariate to the model (see supplementary Tables 7). In the replication set, methylation levels of *BRSK2* CpG3 ($\beta = -0.00$, $p = .889$, OR 1.00), CpG4 ($\beta = -0.01$, $p = .667$, OR0.99) and CpG5 ($\beta = 0.00$, $p = .866$, OR 1.00) were not significantly associated with PTSD status at 3-months post-rape (see supplementary Table 8).

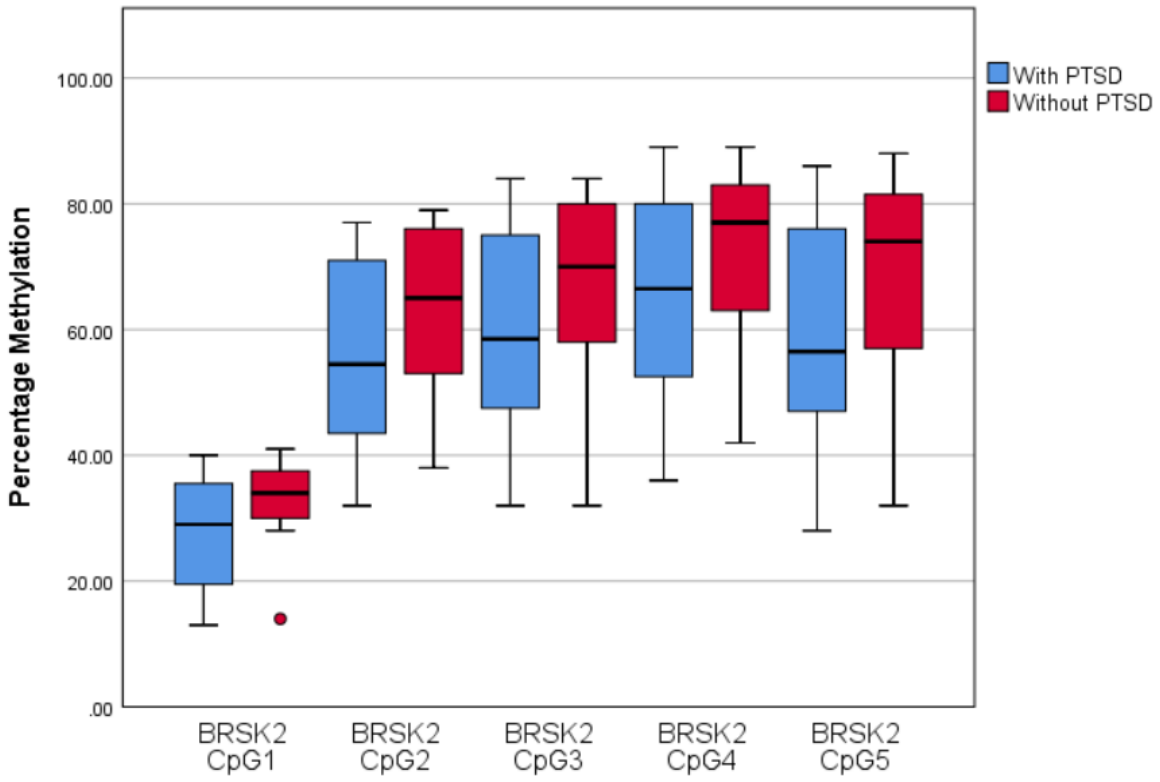


Figure 1: Boxplots indicating methylation levels between participants with and without PTSD for the five CpG sites in the *BRSK2* region found to be associated with PTSD at 3-months post-rape in the epigenome-wide association study.

Validation and replication sets: Differential methylation of *ADCYAP1* in relation to PTSD status at 3-months post-rape

The *ADCYAP1* region (chr18:905177-905180) identified from the EWAS included only two differentially methylated CpG sites (CpG1 – cg22388954, CpG2 – cg11773720) which both showed increased methylation in participants with PTSD (see Figure 2). Based on prior findings, DNA methylation of these CpG sites in blood were not correlated with DNA methylation in brain tissue (Supplementary Figures 17a and 17b) [58]. EpiTYPER signals for *ADCYAP1* CpG1 and CpG2 were combined for analysis, due to their proximity to each other (see supplementary Table 9 for the genomic coordinates and sequence for CpG1 and CpG2).

Baseline age, HIV status, BMI, smoking status, childhood trauma score, lifetime trauma, alcohol use, depression and medication use were not associated with *ADCYAP1* methylation at 3-months post-rape in the validation or replication sets (see supplementary Tables 5 & 6). In the validation set, methylation levels of *ADCYAP1* CpG1&2 (β = -0.09, p =.382, OR 0.92) was not significantly associated with PTSD status at 3-months post-rape (see supplementary Tables 7). In the replication set, methylation levels of *ADCYAP1* CpG1&2 (β =

-0.06, p =.639, OR 0.94) was also not significantly associated with PTSD status at 3-months post-rape (see supplementary Table 8).

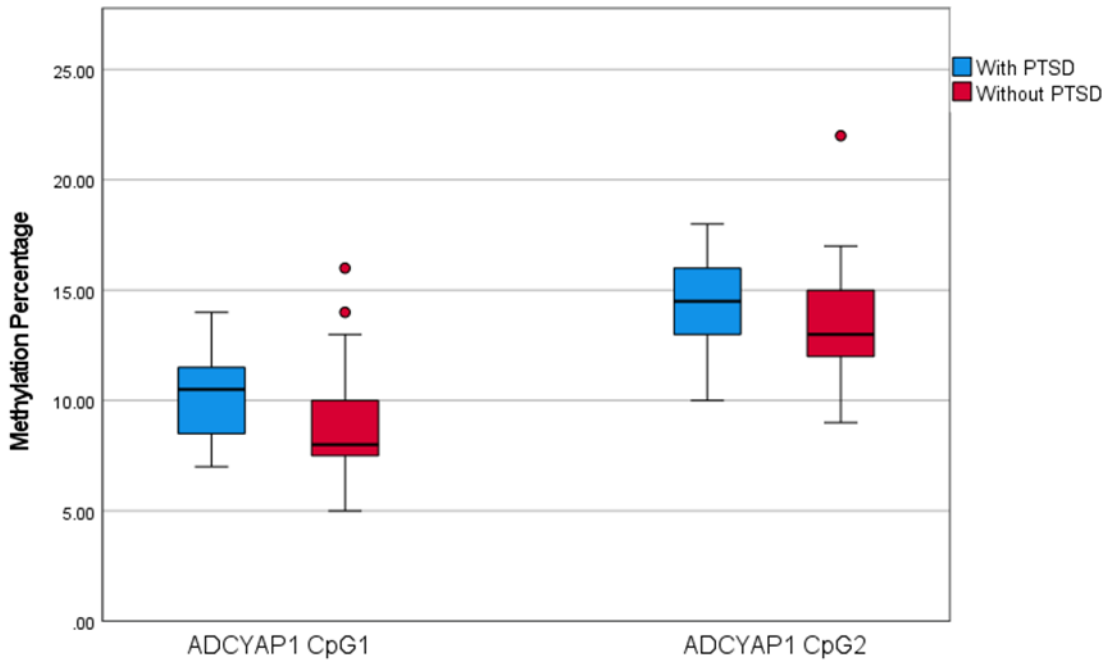


Figure 2: Boxplots indicating methylation levels between participants with and without PTSD for the two CpG sites in the *ADCYAP1* region found to be associated with PTSD in the epigenome-wide methylation study.

Agreement between the MethylationEPIC array and EpiTYPER

Large positive correlations were found when comparing the MethylationEPIC array and EpiTYPER methylation levels for *BRSK2* CpG3 (r = .881, p < .000), CpG4 (r = .900, p < .000) and CpG5 (r = .831, p = .831) at 3-months post-rape. Small, non-significant correlations were found when comparing the MethylationEPIC array and EpiTYPER methylation levels for *ADCYAP1* CpG1&2 (r = .254, p > .05; see Supplementary Tables 10 & 11).

Replication of previous candidate gene and EWAS findings

Differential methylation of five CpG sites previously investigated was replicated in this EWAS study, prior to correction for multiple testing (see Supplementary Table 12 and 13). These sites were located in the *HTR3A* (chr11:113846004, cg20621129, p = .028) [64], *AHRR* (two CpG

sites: chr5:373378, cg05575921, p = .033; chr5:377358, cg26703534, p = .031) [22], *DUSP22* (chr6:291882, cg21548813, p = .032) [15] and *TPR* (chr1:186344558, cg24577137, p = .0008) genes [13]. Since decreased methylation of *AHRR* is strongly linked to smoking [22] we investigated the link between smoking and *AHRR* methylation (based on the values obtained from our EWAS) and found decreased *AHRR* methylation in smokers (M = 78.91, SD = 14.95, n = 5) compared to non-smokers (M = 93.88, SD = 1.45, n = 42) at cg05575921 (z = -2.92, p = .001).

Longitudinal relationship between *BRSK2, ADCYAP1,* PTSD scores and confounding variables

Baseline childhood trauma, alcohol use and depression were associated with PTSD scores at one or more timepoints. Baseline childhood trauma and lifetime trauma were associated with *BRSK2* methylation at one or more timepoints. Baseline HIV status was associated with *ADCYAP1* methylation at 3-months post-rape (see supplementary Table 14).

Longitudinal change in PTSD symptom scores

The mean PTSD scores at baseline, 3-months and 6-monhs, stratified by group (discovery/validation, replication, combined) are presented in Figure 3. There were no significant differences between the discovery/validation set and the replication set for baseline (z = -0.79, p = .431), 3-month (z = -1.37, p = .172) and 6-month (z = -0.15, p = .883) PTSD scores.

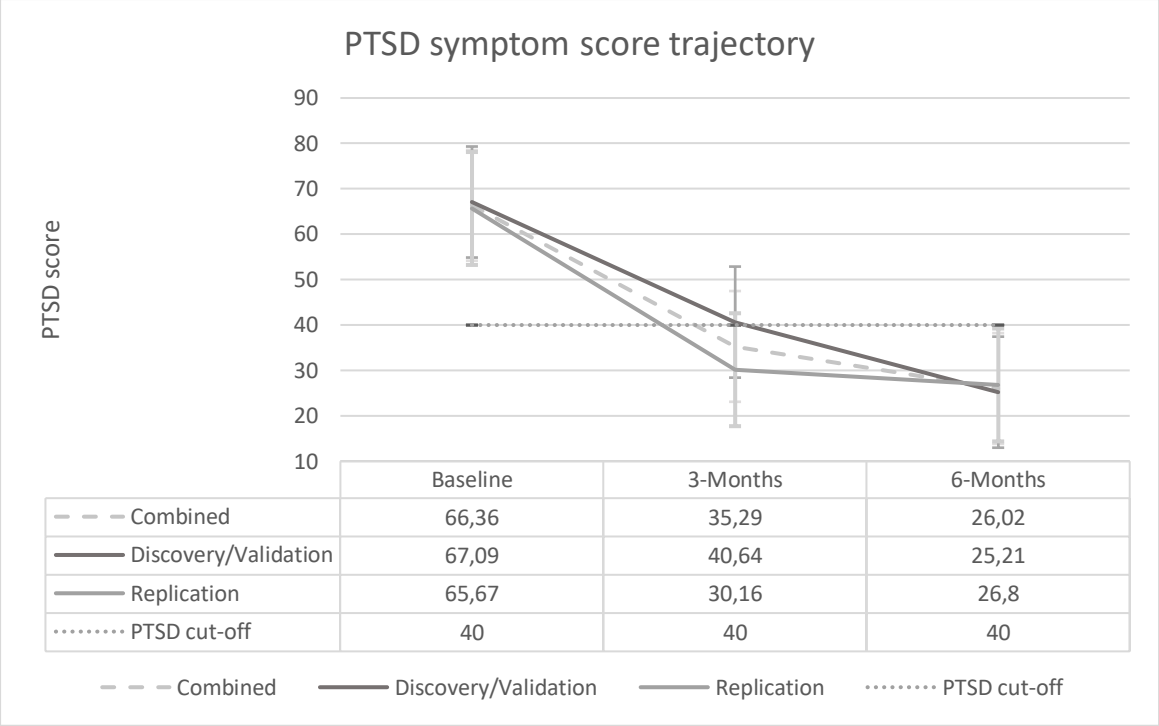


Figure 3: PTSD symptom score trajectories over 6 months for the discovery/validation set, replication set and combined set

Combined set: Baseline *BRSK2* and *ADCYAP1* methylation levels and longitudinal change in PTSD scores

Table 5 presents the results of the mixed regression models investigating baseline *BRSK2* and *ADCYAP1* methylation as predictors of change in PTSD symptom scores over time. Decreased baseline *BRSK2* CpG3, CpG4 and CpG5 methylation was a significant predictor of increased PTSD symptom scores at 3-months (CpG3 *ß* = -0.39, p <.001, CpG4 *ß* = -0.33, p =.005, CpG5 *ß* = -0.27, p =.009) and 6-months (CpG3 *ß* = -0.49, p <.001, CpG4 *ß* = -0.44, p <.001, CpG5 *ß* = -0.38, p <.001) post-rape. However, the relationships between *BRSK2* CpG3, CpG4 and CpG5 methylation and PTSD scores at 3-months and 6-months were no longer significant when covariates were added to the models.

Increased baseline *ADCYAP1* CpG1&2 methylation was a significant predictor of increased PTSD scores at baseline (*ß* = 5.34, p <.001) and decreased PTSD scores at 6-months (*ß* = -3.52, p =.004) post-rape, but the associations were no longer significant when covariates were added to the model.

Table 5: *Summary statistics of the mixed regression models investigating baseline BRSK2 and ADCYAP1 methylation as predictors of change in PTSD symptoms scores over time*

Model		ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	Baseline *BRSK2* CpG3 methylation						
1A	Baseline x CpG3	0.07	0.10	0.71	.482	-0.13	0.27
	3-months x CpG3	-0.39	0.10	-3.81	.0002*	-0.60	-0.19
	6-months x CpG3	-0.49	0.10	-4.76	.000004*	-0.70	-0.29
1B	Baseline x CpG3	-0.12	0.10	-1.17	.247	-0.33	0.08
	3-months x CpG3	-0.16	0.15	-1.09	.276	-0.45	0.13
	6-months x CpG3	-0.12	0.15	-0.81	.418	-0.41	0.17
	Baseline x Childhood trauma	1.54	0.49	3.13	.002*	0.57	2.51
	3-months x Childhood trauma	1.21	0.66	1.84	.069	-0.09	2.52
	6-months x Childhood trauma	-035	0.66	-0.53	.598	-1.65	0.96
	Baseline x Alcohol consumption	-1.36	0.76	-1.79	.077	-2.87	0.15
	3-months x Alcohol consumption	-0.96	1.23	-0.78	.438	-3.40	1.48
	6-months x Alcohol consumption	-1.65	1.23	-1.35	.181	-4.09	0.78
	Baseline x Depression	0.73	0.14	5.29	.0000008*	0.46	1.01
	3-months x Depression	0.00	0.22	0.00	1.00	-0.45	0.45
	6-months x Depression	0.49	0.22	2.19	.031*	0.05	0.93
1C	Baseline x CpG3	-0.10	0.10	-1.01	.314	-0.31	0.10
	3-months x CpG3	-0.15	0.15	-1.03	.305	-0.44	0.14
	6-months x CpG3	-0.10	0.15	-0.68	.496	-0.39	0.19
	Baseline x Childhood trauma	1.12	0.52	2.45	.016*	0.24	2.30
	3-months x Childhood trauma	1.12	0.73	1.54	.126	-0.32	2.56
	6-months x Childhood trauma	-0.67	0.72	-0.93	.355	-2.10	0.76
	Baseline x Alcohol consumption	-1.40	0.76	-1.85	.067	-2.90	0.10
	3-months x Alcohol consumption	-0.98	1.24	-0.79	.432	-3.43	1.48
	6-months x Alcohol consumption	-1.70	1.23	-1.38	.170	-4.13	0.73
	Baseline x Depression	0.77	0.14	5.50	.0000003*	0.49	1.04
	3-months x Depression	0.01	0.23	0.06	.952	-0.44	0.47
	6-months x Depression	0.53	0.23	2.35	.021*	0.08	0.98
	Baseline x Lifetime trauma	2.06	1.36	1.51	.134	-0.65	4.76
	3-months x Lifetime trauma	0.79	2.24	0.35	.727	-3.66	5.23
	6-months x Lifetime trauma	2.47	2.22	1.11	.270	-1.95	6.88
	Baseline *BRSK2* CpG4 methylation						
2A	Baseline x CpG4	0.07	0.11	0.59	.558	-0.16	0.29
	3-months x CpG4	-0.33	0.12	-2.85	.005*	-0.56	-0.10
	6-months x CpG4	-0.44	0.12	-3.83	.0002*	-0.67	-0.21
2B	Baseline x CpG4	-0.08	0.12	-0.70	.486	-0.31	0.15
	3-months x CpG4	-0.14	0.15	-0.93	.357	-0.44	0.16
	6-months x CpG4	-0.19	0.15	-1.29	.201	-0.49	0.10
	Baseline x Childhood trauma	1.44	0.51	2.84	.005*	0.43	2.44

	3-months x Childhood trauma	1.20	0.70	1.73	.086	-0.17	2.58
	6-months x Childhood trauma	-0.09	0.69	-0.12	.902	-1.45	1.28
	Baseline x Alcohol consumption	-1.41	0.77	-1.84	.069	-2.93	0.11
	3-months x Alcohol consumption	-1.00	1.23	-0.81	.421	-3.44	1.45
	6-months x Alcohol consumption	-1.60	1.22	-1.32	.191	-4.02	0.82
	Baseline x Depression	0.71	0.14	5.08	.000002*	0.43	0.98
	3-months x Depression	-0.01	0.23	-0.05	.963	-0.46	0.44
	6-months x Depression	0.53	0.22	2.40	.018*	0.09	0.98
2C	Baseline x CpG4	-0.04	0.12	-0.36	.720	-0.27	0.19
	3-months x CpG4	-0.12	0.16	-0.75	.458	-0.42	0.19
	6-months x CpG4	-0.16	0.15	-1.01	.314	-0.46	0.15
	Baseline x Childhood trauma	1.20	0.53	2.24	.027*	0.14	2.25
	3-months x Childhood trauma	1.17	0.77	1.53	.130	-0.35	2.69
	6-months x Childhood trauma	-0.34	0.76	-0.45	.654	-1.84	1.16
	Baseline x Alcohol consumption	-1.47	0.76	-1.93	.056	-2.99	0.04
	3-months x Alcohol consumption	-1.03	1.24	-0.83	.407	-3.49	1.43
	6-months x Alcohol consumption	-1.67	1.22	-1.37	.175	-4.09	0.75
	Baseline x Depression	0.74	0.14	5.28	.0000008	0.46	1.02
	3-months x Depression	0.00	0.23	0.01	.996	-0.45	0.46
	6-months x Depression	0.57	0.22	2.52	.013*	0.12	1.01
	Baseline x Lifetime trauma	2.11	1.39	1.52	.132	-0.65	4.88
	3-months x Lifetime trauma	0.71	2.27	0.32	.753	-3.79	5.22
	6-months x Lifetime trauma	2.19	2.23	0.98	.329	-2.24	6.62
	Baseline *BRSK2* CpG5 methylation						
3A	Baseline x CpG5	0.16	0.10	1.60	.112	-0.04	0.35
	3-months x CpG5	-0.27	0.10	-2.66	.009*	-0.47	-0.07
	6-months x CpG5	-0.38	0.10	-3.73	.0003*	-0.58	-0.18
3B	Baseline x CpG5	-0.06	0.10	-0.57	.573	-0.25	0.14
	3-months x CpG5	-0.12	0.14	-0.84	.405	-0.39	0.16
	6-months x CpG5	-0.06	0.14	-0.44	.657	-0.33	0.21
	Baseline x Childhood trauma	1.60	0.50	3.21	.002*	0.61	2.58
	3-months x Childhood trauma	1.33	0.66	2.01	.047*	0.02	2.65
	6-months x Childhood trauma	-0.27	0.66	-0.41	.683	-1.58	1.04
	Baseline x Alcohol consumption	-1.44	0.77	-1.88	.064	-2.96	0.08
	3-months x Alcohol consumption	-1.01	1.23	-0.82	.412	-3.46	1.43
	6-months x Alcohol consumption	-1.72	1.23	-1.41	.163	-4.16	0.71
	Baseline x Depression	0.72	0.14	5.13	.000002*	0.44	1.00
	3-months x Depression	0.00	0.23	0.01	.991	-0.45	0.45
	6-months x Depression	0.48	0.22	2.14	.035*	0.03	0.93
3C	Baseline x CpG5	-0.03	0.10	-0.29	.774	-0.23	0.17
	3-months x CpG5	-0.10	0.14	-0.71	.477	-038	0.18
	6-months x CpG5	-0.03	0.14	-0.22	.827	-0.31	0.25
	Baseline x Childhood trauma	1.34	0.53	2.56	.012*	0.30	2.39
	3-months x Childhood trauma	1.28	0.73	1.74	.085	-0.18	2.73

	6-months x Childhood trauma	-0.58	0.73	-0.80	.425	-2.03	0.86
	Baseline x Alcohol consumption	-1.49	0.76	-1.96	.054	-3.01	0.02
	3-months x Alcohol consumption	-1.04	1.24	-0.84	.402	-3.50	1.41
	6-months x Alcohol consumption	-1.78	1.22	-1.46	.148	-4.21	0.65
	Baseline x Depression	0.75	0.14	5.33	.0000007*	0.47	1.03
	3-months x Depression	0.01	0.23	0.06	.950	-0.44	0.47
	6-months x Depression	0.52	0.23	2.29	.024*	0.07	0.97
	Baseline x Lifetime trauma	2.12	1.39	1.53	.129	-0.63	4.88
	3-months x Lifetime trauma	0.74	2.26	0.33	0.75	-3.74	5.22
	6-months x Lifetime trauma	2.52	2.23	1.13	.262	-1.92	6.96
	Baseline *ADCYAP1* CpG1&2 methylation						
4A	Baseline x CpG1&2	5.34	1.02	5.26	.0000009*	3.33	7.36
	3-months x CpG1&2	-1.03	1.12	-0.92	.360	-3.25	1.19
	6-months x CpG1&2	-3.52	1.18	-2.97	.004*	-5.86	-1.17
4B	Baseline x CpG1&2	-0.73	0.88	-0.83	.407	-2.47	1.01
	3-months x CpG1&2	2.75	1.39	1.98	.050	-0.00	5.50
	6-months x CpG1&2	1.66	1.39	1.20	.235	-1.10	4.41
	Baseline x Childhood trauma	1.84	0.47	3.95	.0001*	0.92	2.76
	3-months x Childhood trauma	1.11	0.59	1.88	.062	-0.05	2.26
	6-months x Childhood trauma	-0.29	0.59	-0.49	.625	-1.45	0.87
	Baseline x Alcohol consumption	-1.48	0.77	-1.93	.057	-3.01	0.05
	3-months x Alcohol consumption	-1.43	1.22	-1.18	.242	-3.85	0.98
	6-months x Alcohol consumption	-1.91	1.21	-1.57	.120	-4.32	0.51
	Baseline x Depression	0.73	0.14	5.29	.0000008*	0.46	1.01
	3-months x Depression	-0.13	0.22	-0.59	.554	-0.56	0.30
	6-months x Depression	0.42	0.22	1.96	.052	-0.00	0.85
4C	Baseline x CpG1&2	-0.54	0.89	-0.61	.541	-2.30	1.22
	3-months x CpG1&2	2.76	1.42	1.95	.055	-0.05	5.57
	6-months x CpG1&2	1.59	1.41	1.13	.263	-1.22	4.40
	Baseline x Childhood trauma	1.87	0.47	4.01	.0001*	0.95	2.80
	3-months x Childhood trauma	1.06	0.59	1.79	.076	-0.11	2.24
	6-months x Childhood trauma	-0.36	0.59	-0.60	.550	-1.53	0.82
	Baseline x Alcohol consumption	-1.44	0.76	-1.88	.063	-2.96	0.08
	3-months x Alcohol consumption	-1.43	1.22	-1.17	.247	-3.86	1.01
	6-months x Alcohol consumption	-1.91	1.22	-1.56	.121	-4.34	0.52
	Baseline x Depression	0.74	0.14	5.33	.0000006*	0.46	1.01
	3-months x Depression	-0.13	0.22	-0.61	.546	-0.56	0.30
	6-months x Depression	0.42	0.22	1.92	.058	-0.01	0.85
	Baseline x HIV status	-4.51	3.54	-1.27	.206	-11.55	2.53
	3-months x HIV status	-0.39	5.65	-0.07	.945	-11.61	10.84
	6-months x HIV status	1.24	5.65	0.22	.827	-9.99	12.46

Abbreviations: confidence interval (CI); brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; adenylate cyclase activating polypeptide 1 *(ADCYAP1)*

Combined set: longitudinal change in ADCYAP1 and BRSK2 methylation levels in relation to longitudinal change in PTSD scores

Table 6 presents the results of the mixed regression models investigating change in *BRSK2* and *ADCYAP1* methylation over time as predictors of change in PTSD symptom scores over time. Decreased *BRSK2* CpG3 (ß = -0.39, $p < .001$), CpG4 (ß = -0.36, $p = .001$) and CpG5 (ß = -0.32, $p = .001$) methylation at 3-months post-rape was associated with increased PTSD scores at 3-months post-rape. Decreased *BRSK2* CpG3 (ß = -0.49, $p < .001$), CpG4 (ß = -0.46, $p < .001$) and CpG5 (ß = -0.43, $p < .001$) methylation at 6-months post-rape was also associated with increased PTSD scores at 6-months post-rape. None of the associations remained significant after adding the covariates to the models, with the exception of BRSK2 CpG3 (ß = -0.30, $p = .049$).

Increase baseline *ADCYAP1* CpG1&2 methylation was associated with increased PTSD scores at baseline (ß = 4.67, $p < .001$) while decreased *ADCYAP1* CpG1&2 methylation at 3-months (ß = -2.61, $p = .001$) and 6-months (ß = -5.01, $p < .001$) was associated with increased PTSD scores at 3-months and 6-months post-rape, consecutively. The associations were no longer significant when the covariates were added to the model.

Table 6: *Summary statistics of the mixed regression models investigating change in BRSK2 and ADCYAP1 methylation over time as predictors of change in PTSD symptoms scores over time*

Model		ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	Baseline *BRSK2* CpG3 methylation						
1A	Baseline x CpG3	0.07	0.10	0.71	.482	-0.13	0.27
	3-months x CpG3	-0.39	0.10	-3.81	.0002*	-0.60	-0.19
	6-months x CpG3	-0.49	0.10	-4.76	.000004*	-0.70	-0.29
1B	Baseline x CpG3	-0.16	0.10	-1.60	.111	-0.37	0.04
	3-months x CpG3	-0.31	0.15	-2.06	.041*	-0.60	-0.01
	6-months x CpG3	-0.15	0.14	-1.02	.308	-0.44	0.14
	Baseline x Childhood trauma	1.43	0.49	2.93	.004*	0.47	2.40
	3-months x Childhood trauma	1.39	0.66	2.11	.037*	0.08	2.70
	6-months x Childhood trauma	-0.51	0.66	-0.78	.436	-1.81	0.78
	Baseline x Alcohol consumption	-1.31	0.76	-1.72	.088	-2.81	0.20
	3-months x Alcohol consumption	-0.86	1.22	-0.71	.481	-3.28	1.56
	6-months x Alcohol consumption	-1.60	1.24	-1.29	.199	-4.06	0.86
	Baseline x Depression	0.74	0.14	5.34	.0000006*	0.46	1.01
	3-months x Depression	0.06	0.22	0.26	.793	-0.38	0.50
	6-months x Depression	0.50	0.23	2.16	.034*	0.04	0.97
1C	Baseline x CpG3	-0.15	0.10	-1.42	.157	-0.35	0.06
	3-months x CpG3	-0.30	0.15	-1.99	.049*	-0.60	-0.00
	6-months x CpG3	-0.12	0.15	-0.80	.423	-0.41	0.17
	Baseline x Childhood trauma	1.18	0.52	2.89	.024*	0.16	2.21
	3-months x Childhood trauma	1.34	0.73	1.84	.068	-0.10	2.78
	6-months x Childhood trauma	-0.82	0.73	-1.12	.263	-2.25	0.62
	Baseline x Alcohol consumption	-1.35	0.75	-1.79	.077	-2.84	0.15
	3-months x Alcohol consumption	-0.88	1.23	-0.71	.477	-3.31	1.56
	6-months x Alcohol consumption	-1.66	1.24	-1.34	.184	-4.11	0.80
	Baseline x Depression	0.77	0.14	5.55	.0000002*	0.49	1.05
	3-months x Depression	0.07	0.23	0.31	.761	-0.38	0.52
	6-months x Depression	0.53	0.23	2.27	.026*	0.07	1.00
	Baseline x Lifetime trauma	2.02	1.36	1.48	.141	-0.68	4.72
	3-months x Lifetime trauma	0.57	2.22	0.26	.797	-3.84	4.98
	6-months x Lifetime trauma	2.28	2.27	1.01	.317	-2.23	6.79

Model		ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	Baseline *BRSK2* CpG4 methylation						
2A	Baseline x CpG4	0.03	0.11	0.32	.749	-0.17	0.24
	3-months x CpG4	-0.36	0.11	-3.40	.001*	-0.57	-0.15
	6-months x CpG4	-0.46	0.11	-4.92	.00003*	-0.68	-0.25
2B	Baseline x CpG4	-0.13	0.11	-1.21	.230	-0.35	0.84
	3-months x CpG4	-0.30	.15	-2.04	.043*	-0.59	-0.01

	6-months x CpG4	-0.22	0.15	-1.49	.138	-0.51	0.07
	Baseline x Childhood trauma	1.36	0.49	2.75	.007*	0.38	2.34
	3-months x Childhood trauma	1.48	0.69	2.14	.034*	0.11	2.84
	6-months x Childhood trauma	-0.28	0.69	-0.40	.689	-1.64	1.09
	Baseline x Alcohol consumption	-1.35	0.76	-1.77	.080	-2.87	0.16
	3-months x Alcohol consumption	-0.85	1.22	-0.70	.488	-3.27	1.57
	6-months x Alcohol consumption	-1.59	1.22	-1.30	.197	-4.02	0.84
	Baseline x Depression	0.72	0.14	5.15	.000001*	0.44	0.99
	3-months x Depression	0.07	0.22	0.32	.751	-0.37	0.52
	6-months x Depression	0.55	0.23	2.38	.020*	0.098	1.01
2C	Baseline x CpG4	-0.10	0.11	-0.88	.379	-0.32	0.12
	3-months x CpG4	-0.28	0.15	-1.88	.062	-0.58	0.14
	6-months x CpG4	-0.19	0.15	-1.25	.215	-0.48	0.11
	Baseline x Childhood trauma	1.12	0.52	2.14	.035*	0.08	2.15
	3-months x Childhood trauma	1.46	0.76	1.93	.056	-0.04	2.96
	6-months x Childhood trauma	-0.53	0.76	-0.70	.488	-2.03	0.97
	Baseline x Alcohol consumption	-1.41	0.76	-1.85	.067	-2.92	0.10
	3-months x Alcohol consumption	-0.87	1.23	-0.71	.478	-3.31	1.56
	6-months x Alcohol consumption	-1.64	1.22	-1.34	.183	-4.08	0.79
	Baseline x Depression	0.75	0.14	5.34	.0000006*	0.47	1.02
	3-months x Depression	0.79	0.23	0.35	.730	-0.37	0.53
	6-months x Depression	0.58	0.23	2.48	.015*	0.12	1.04
	Baseline x Lifetime trauma	2.00	1.39	1.44	.154	-0.76	4.75
	3-months x Lifetime trauma	0.49	2.22	0.22	.826	-3.93	4.91
	6-months x Lifetime trauma	2.12	2.25	0.95	.345	-2.33	6.59

Model		*ß*	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	Baseline *BRSK2* CpG5 methylation						
3A	Baseline x CpG5	0.10	0.09	1.07	.285	-0.08	0.28
	3-months x CpG5	-0.32	0.09	-3.40	.001*	-0.51	-0.14
	6-months x CpG5	-0.43	0.10	-4.42	.00002*	-0.62	-0.24
3B	Baseline x CpG5	-0.11	0.10	-1.10	.275	-0.30	0.09
	3-months x CpG5	-0.25	0.14	-1.81	.073	-0.53	0.02
	6-months x CpG5	-0.15	0.13	-1.11	.269	-0.42	0.12
	Baseline x Childhood trauma	1.43	0.49	2.91	.004*	0.46	2.40
	3-months x Childhood trauma	1.46	0.67	2.17	.032*	0.13	2.78
	6-months x Childhood trauma	-0.35	0.66	-0.54	.593	-1.65	0.95
	Baseline x Alcohol consumption	-1.37	0.76	-1.79	.076	-2.88	0.15
	3-months x Alcohol consumption	-0.98	1.23	-0.80	.429	-3.41	1.46
	6-months x Alcohol consumption	-1.62	1.23	-1.32	.190	-4.06	0.82
	Baseline x Depression	0.72	0.14	5.18	.000001*	0.45	1.00
	3-months x Depression	0.05	0.22	0.21	.834	-0.40	0.49
	6-months x Depression	0.52	0.23	2.26	.027*	0.06	0.98

3C	Baseline x CpG5	-0.08	0.10	-0.81	.417	-0.27	0.11
	3-months x CpG5	-0.24	0.14	-1.68	.095	-0.51	0.04
	6-months x CpG5	-0.12	0.14	-0.86	.390	-0.39	0.15
	Baseline x Childhood trauma	1.19	0.52	2.28	.024*	0.16	2.22
	3-months x Childhood trauma	1.41	0.74	1.91	.058	-0.05	2.87
	6-months x Childhood trauma	-0.62	0.73	-0.86	.391	-2.06	0.81
	Baseline x Alcohol consumption	-1.42	0.76	-1.87	.065	-2.93	0.88
	3-months x Alcohol consumption	-0.99	1.23	-0.81	.423	-3.45	1.46
	6-months x Alcohol consumption	-1.68	1.23	-1.37	.175	-4.12	0.76
	Baseline x Depression	0.75	0.14	5.37	.0000005*	0.48	1.03
	3-months x Depression	0.06	0.23	0.26	.800	-0.40	0.51
	6-months x Depression	0.55	0.23	2.36	.020*	0.09	1.01
	Baseline x Lifetime trauma	2.03	1.38	1.47	.145	-0.71	4.77
	3-months x Lifetime trauma	0.62	2.24	0.28	.781	-3.82	5.06
	6-months x Lifetime trauma	2.23	2.26	0.98	.328	-2.27	6.72

Model		*ß*	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	Baseline *ADCYAP1* CpG1&2 methylation						
4A	Baseline x CpG1&2	4.67	0.92	5.10	.000001*	2.86	6.49
	3-months x CpG1&2	-2.61	0.80	-3.26	.001*	-4.20	-1.02
	6-months x CpG1&2	-5.01	1.12	-4.48	.00002*	-7.23	-2.80
4B	Baseline x CpG1&2	-1.32	0.83	-1.16	.113	-2.97	0.32
	3-months x CpG1&2	-1.46	0.92	-1.59	.116	-3.29	0.37
	6-months x CpG1&2	-0.44	1.28	-0.34	.734	-2.97	2.10
	Baseline x Childhood trauma	1.77	0.47	3.76	.0003*	0.84	2.70
	3-months x Childhood trauma	1.44	0.61	2.37	.019*	0.24	2.63
	6-months x Childhood trauma	-0.19	0.60	-0.31	.757	-1.37	1.00
	Baseline x Alcohol consumption	-1.41	0.77	-1.84	.068	-2.93	0.11
	3-months x Alcohol consumption	-1.27	1.24	-1.02	.309	-3.73	1.19
	6-months x Alcohol consumption	-1.74	1.22	-1.42	.158	-4.17	0.69
	Baseline x Depression	0.74	0.14	5.35	.0000006*	0.46	1.01
	3-months x Depression	-0.03	0.22	-0.16	.876	-0.46	0.40
	6-months x Depression	0.46	0.21	2.14	.035*	0.03	0.88
4C	Baseline x CpG1&2	-1.12	0.83	-1.35	.182	-2.78	0.54
	3-months x CpG1&2	-1.54	0.93	-1.66	.100	-3.38	0.30
	6-months x CpG1&2	-0.48	1.29	-0.37	.712	-3.03	2.08
	Baseline x Childhood trauma	1.80	0.47	3.84	.0002*	0.87	2.73
	3-months x Childhood trauma	1.37	0.61	2.23	.027*	0.16	2.58
	6-months x Childhood trauma	-0.26	0.61	-0.42	.675	-1.46	0.95
	Baseline x Alcohol consumption	-1.38	0.76	-1.81	.074	-2.90	0.17
	3-months x Alcohol consumption	-1.29	1.25	-1.04	.302	-3.77	1.18
	6-months x Alcohol consumption	-1.76	1.23	-1.43	.157	-4.21	0.69
	Baseline x Depression	0.74	0.14	5.40	.0000005*	0.47	1.01
	3-months x Depression	-0.04	0.22	-0.20	.844	-0.48	0.39

6-months x Depression	0.45	0.22	2.08	.040*	0.02	0.88
Baseline x HIV status	-4.28	3.53	-1.21	.229	-11.30	2.74
3-months x HIV status	2.16	5.78	0.37	.709	-9.32	13.65
6-months x HIV status	1.86	5.60	0.33	.740	-9.27	12.99

Abbreviations: confidence interval (CI); brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; adenylate cyclase activating polypeptide 1 *(ADCYAP1)*.

DISCUSSION

In this study we identified one DMP (cg01700569) and thirty-four DMRs associated with PTSD at 3-months post-rape on an epigenome-wide level. We investigated two DMRs in the *BRSK2* and *ADCYAP1* genes further. We were able to validate, but not replicate, the *BRSK2* CpG5 finding, which confirmed that participants with PTSD at 3-months post-rape showed decreased *BRSK2* methylation compared to those without PTSD. We also found that decreased baseline *BRSK2* CpG3, CpG4 and CpG5 methylation was associated with increased PTSD scores at 3- and 6-months post-rape. Decreased *BRSK2* methylation at 3-months and 6-months post-rape was associated with increased PTSD scores at the same time-points. However, the relationship between decreased *BRSK2* CpG3 methylation at 3-months post-rape and increased PTSD scores at 3-months post-rape was the only association that remained significant after covariates were added to the models.

We were unable to validate or replicate the *ADCYAP1* CpG1&2 findings. We found that decreased baseline *ADCYAP1* CpG1&2 methylation was associated with increased PTSD scores at 6-months post-rape. Decreased *ADCYAP1* methylation at 3-months and 6-months post-rape was also associated with increased PTSD scores at the same timepoints, while decreased baseline *ADCYAP1* CpG1&2 methylation was associated with decreased PTSD scores at baseline. The findings did not remain significant after PTSD covariates were added to the models.

Decreased methylation of the *BRSK2* paralog, *BRSK1*[65], has been associated with a PTSD diagnosis in a prior EWAS [14]. *BRSK1* and *BRSK2* share a 68% overlap in genetic sequence, both are highly expressed in the brain and decreased expression of both has been linked to disorganised presynaptic vesicle formation, uncoordinated release and reuptake of neurotransmitters, altered axonal development and abnormal neuronal polarisation in animal studies [65–70]. In human studies, a *BRSK2* polymorphism (rs1881509) has been associated with heroin dependence [66] and functional variants of *BRSK2* have been associated with autism spectrum disorder, cognitive impairment, intellectual disability and speech delays [71,72].

Genes *BRSK1* and *BRSK2* are expressed most strongly in the cerebellum and the hippocampus [66]. The hippocampus is closely linked to PTSD since it is involved in memory consolidation [73]. When memories are not consolidated into autobiographical memory networks, they may involuntarily resurface (e.g. flashbacks, intrusions, nightmares and dissociation) and activate the limbic system which induces the fight-or-flight response [74]. Differential methylation and expression of *BRSK2* may also alter the expression of neurotransmitters

previously found to be associated with PTSD (e,g. norepinephrine, epinephrine, dopamine and serotonin) through altered presynaptic vesicle and synaptic cleft development [75,76].

In addition to its function in the brain, *BRSK1* and *BRSK2* have been linked to metabolic processes and glucose homeostasis [77,78]. Animal studies have found increased expression of *BRSK1* and *BRSK2* in pancreatic cells and knockdown of *BRSK2* resulted in a significant increase in serum insulin levels [77,78]. In a human study, *BRSK2* was found to be highly expressed in human pancreatic insulin producing B-cells and activation of *BRSK2* was linked to reduced insulin secretion [78]. An EWAS found that participants with type 1 diabetes and neuropathy showed decreased methylation at four CpG sites in the *BRSK2* gene compared to participants with type 1 diabetes without neuropathy [79].

The *BRSK2* CpG sites investigated in this study were located in intron 4 of the gene. The function of methylation in gene bodies is not well established, but methylation is abundant in these regions and is generally positively correlated with expression [80]. Assuming the latter, we can hypothesise that decreased methylation of *BRSK2* may contribute to adverse neuronal development, neuronal maintenance and dysregulated blood glucose levels which may explain the increased risk for diabetes and cardiovascular disease observed in prior PTSD studies [81,82]. The relationship between *BRSK2* methylation and adverse neuronal development and maintenance is further supported by prior findings of a high correlation between *BRSK2* blood methylation and methylation in brain tissue [58].

We investigated *ADCYAP1* since its protein product, PACAP, has been identified as a master regulator of the HPA-axis and the stress response [83]. The highest concentration of PACAP in the brain is found in the hypothalamus [84]. PACAP binding in the hypothalamus triggers the release of corticotrophin-releasing hormone (CRH) and signals the activation of the stress response [83]. In the adrenal medulla, PACAP binding to PAC1R (product of *ADCYAP1R1*) stimulates the release of catecholamines as part of the sympathetic nervous system (SNS) [85]. PACAP binding to PACR1 in preganglionic neurons triggers the release of phenylethanolamine-N-methyltransferase (PNMT) and tyrosine hydroxylase (TH) in effector organs of the SNS. PNMT and TH are catecholamine-synthesising enzymes and sustain the release of catecholamines in the effector organs during the stress response [85].

Researchers investigating PACAP/*ADCYAP1* and PACR1/*ADCYAP1R1* in relation to PTSD in a predominantly African American sample with a mixture of trauma types found that, in women more than men, increased PACAP blood levels were associated with increased PTSD symptom severity and an increased acoustic startle reflex response [61,86]. They also found that women carrying the *ADCYAP1R1* rs2267735 CC genotype showed decreased *ADCYAP1R1* mRNA expression, increased PTSD symptom severity, increased dark-enhanced startle

response and increased amygdala and hippocampal activity in response to viewing threatening face stimuli [61–63,86]. In both men and women, increased methylation of *ADCYAP1R1* was associated with decreased cortical mRNA expression and increased PTSD symptom severity [61,87]. However, the functional effects of *ADCYAP1* and *ADCYAP1R1* seem to be more pronounced in women compared to men [61–63], due to the presence of several estrogen response elements (EREs) in the *ADCYAP1R1* promoter. The CC genotype of rs2267735 has been associated with decreased binding of estrogen receptor alpha to the EREs and decreased expression of *ADCYAP1R1* [88]. The role of estrogen in *ADCYAP1R1* and HPA-axis activity may in part explain why women have an increased risk of PTSD compared to men [35,89].

The two *ADCYAP1* CpG sites investigated in this study are located in a CpG island spanning the 1st intron of the gene. Methylation in CpG islands and in the 1st intron of a gene is generally associated with decreased expression of the gene [90–92]. Our longitudinal findings therefore correspond with prior findings since decreased methylation of *ADCYAP1* is likely to result in increased expression of PACAP and increased PTSD symptom severity [62,63,88,93]. Decreased PACAP is also likely to result in decreased binding to PAC1 and reduced activation of the HPA-axis [83,85].

Based on prior findings, *ADCYAP1* CpG1&2 DNA methylation in blood was not significantly correlated with DNA methylation at the same sites in brain tissue [58]. However, the brain regions investigated did not specifically focus on the region where PACAP is most abundantly expressed i.e., the paraventricular nucleus of the hypothalamus and investigating blood-brain methylation in this region may show different results [37]. It is also likely that expression of PACAP in the endocrine system has a more profound effect on the regulation of the HPA-axis compared to PACAP expression in the brain [37].

In addition to the EWAS, the validation and the replication study, we investigate prior significant findings from published candidate gene studies and EWASs in relation to our findings. We found that, before correction for multiple testing, CpG sites in *HTR3A* [64], *AHRR* [22], *DUSP22* [15] and *TPR* [13] were associated with PTSD. The results from our study are in line with recent results from the largest meta-analysis of PTSD published to date [22], where *AHRR* cg05575921 and cg26703534 were found to exhibit reduced DNA methylation in individuals with PTSD. Decreased *AHRR* methylation at these CpG sites were also associated with decreased kynurenine and kynurenic acid in the same study [22]. Kynurenine ligand binding to aryl hydrocarbon receptors has been associated with the expression of anti-inflammatory genes which may be disrupted by decreased methylation of *AHRR* [22,25]. This may result in increased levels of proinflammatory cytokines and the low-grade inflammatory state often observed in

PTSD [94,95]. Upregulation in kynurenine to restore the imbalance between pro-inflammatory and anti-inflammatory cytokines may also result in reduced levels of serotonin since both kynurenine and serotonin are synthesised from tryptophan [96]. A strong link between decreased *AHRR* methylation and smoking has also been reported in previous studies although some studies have reported a significant relationship between AHRR methylation and PTSD independent of the effect of smoking [22,97–99].

Our findings should be interpreted in light of a number of limitations. First, the EWAS was conducted in a small sample of participants. However, the study was well designed to limit variation between groups. Second, we used DNA extracted from whole blood to measure methylation levels while differential methylation in brain tissue is a more direct approximation of PTSD pathophysiology. However, based on prior findings, we observed that blood-brain methylation was highly correlated in the *BRSK2* CpG sites investigated in this study, but not the *ADCYAP1* CpG sites. Blood is easily accessible and blood biomarkers of PTSD risk may be a more pragmatic approach for personalised treatment of individuals at high risk of developing PTSD following trauma exposure [100]. Finally, DNA methylation in relation to gene expression and/or protein levels was not objectively measured and conclusions related to the functional effects of methylation are speculative.

The study has many strengths. First, all participants were rape-exposed women from similar sociodemographic backgrounds and from the same ethnicity group thus making the sample relatively homogenous. Second, the analyses were robust with a variety of confounding factors controlled for i.e. participants who were pregnant/lactating were excluded, none of the participants were on psychotropic medication and participants were of similar age. Baseline measures of age, HIV status, BMI, smoking, childhood trauma, lifetime trauma, alcohol use and depression were controlled for by matching participants on these variables in the cross-sectional EWAS and including these factors as covariates/confounders in the longitudinal analysis. Third, we attempted to expand the findings of the EWAS by including longitudinal data which allowed us to investigate changes in methylation in relation to PTSD over time. Fourth, investigating the agreement between the results obtained from the two different laboratory methods used (MethylationEpic array and EpiTYPER) also allowed identification of potential bias/variation introduced by the different procedures involved in each method.

In summary, this study provides evidence that differential methylation of genes related to neurogenesis/development, glucose homeostasis and HPA-axis regulation are involved in PTSD development following rape. Our findings are supported by previous research implicating *ADCYAP1/ADCYAP1R1* (especially in women) and *BRSK1/BRSK2* in the development of PTSD. However, replication of our findings is needed to determine if the

differentially methylated regions identified in this study are consistently linked to the development of PTSD. Replication of findings may provide support for *ADCYAP1* and *BRSK2* as biomarkers of PTSD pathogenesis and as potential therapeutic targets.

REFERENCES

1. Breslau N, Troost JP, Bohnert K, Luo Z. Influence of predispositions on post-traumatic stress disorder: Does it vary by trauma severity? *Psychol Med*. 2013;43(2):381-390. doi:10.1017/S0033291712001195

2. Yehuda R, Mcfarlane A, Vermetten E, Lanius R. Post-traumatic stress disorder. *Nat Rev*. 2015;1(October):1-22. doi:10.1038/nrdp.2015.57

3. Elklit A, Christiansen DM. Risk factors for posttraumatic stress disorder in female help-seeking victims of sexual assault. *Violence Vict*. 2013;28(3):552-568. doi:10.1891/0886-6708.09-135

4. Elklit A, Christiansen DM. ASD and PTSD in rape victims. *J Interpers Violence*. 2010;25(8):1470-1488. doi:10.1177/0886260509354587

5. Möller AT, Bäckström T, Söndergaard HP, Helström L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One*. 2014;9(10):1-9. doi:10.1371/journal.pone.0111136

6. Nickerson A, Steenkamp M, Aerka IM, et al. Prospective investigation of mental health following sexual assault. *Depress Anxiety*. 2013;30(5):444-450. doi:10.1002/da.22023

7. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

8. Ryan J, Chaudieu I, Saffery R. Biological underpinnings of trauma and post-traumatic stress disorder: focusing on genetics and epigenetics. *Epigenomics*. 2016;8(11):1553-1569.

9. Nievergelt CM, Ashley-Koch AE, Dalvie S, et al. Genomic Approaches to Posttraumatic Stress Disorder: The Psychiatric Genomic Consortium Initiative. *Biol Psychiatry*. 2018;83(10):831-839. doi:10.1016/j.biopsych.2018.01.020

10. Morrison FG, Miller MW, Logue MW, Assef M, Wolf EJ. DNA methylation correlates of PTSD: Recent findings and technical challenges. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;90(November 2018):223-234. doi:10.1016/j.pnpbp.2018.11.011

11. Mehta D, Klengel T, Conneely KN, et al. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl*

Acad Sci U S A. 2013;110(20):8302-8307. doi:10.1073/pnas.1217750110

12. Hammamieh R, Chakraborty N, Gautam A, et al. Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Transl Psychiatry*. 2017;7(7):e1169. doi:10.1038/tp.2017.129

13. Smith A, Conneely K, Kilaru V, et al. Differential immune system DNA methylation and cytokine regulation in Post-Traumatic Stress Disorder. *Am J Med Genet Paert B Neuropsychiatr Genet*. 2011;156B(6):700-708. doi:10.1109/TMI.2012.2196707.Separate

14. Mehta D, Bruenig D, Carrillo-Roa T, et al. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatr Scand*. 2017;136(5):493-505. doi:10.1111/acps.12778

15. Rutten BPF, Vermetten E, Vinkers CH, et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry*. 2018;23(5):1145-1156. doi:10.1038/mp.2017.120

16. Snijders C, Maihofer AX, Ratanatharathorn A, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clin Epigenetics*. 2020;12(1):1-13. doi:10.1186/s13148-019-0798-7

17. Maddox SA, Kilaru V, Shin J, et al. Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. *Mol Psychiatry*. 2018;23(3):658-665. doi:10.1038/mp.2016.250

18. Uddin M, Aiello AE, Wildman DE, et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2010;107(20):9470-9475. doi:10.1073/pnas.0910794107

19. Chen Y, Li X, Kobayashi I, Tsao D, Mellman T. Expression and methylation in Posttraumatic Stress Disorder and resilience; evidence of the role of odorant receptors. *Psychiatry Res*. 2016;245:36-44. doi:10.1016/j.physbeh.2017.03.040

20. Kuan PF, Waszczuk MA, Kotov R, et al. An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Transl Psychiatry*. 2017;7(6):e1158. doi:10.1038/tp.2017.130

21. Uddin M, Ratanatharathorn A, Armstrong D, Kuan P, Allison E. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics*. 2018;10(12):1585-1601.

22. Smith A, Ratanatharathorn A, Maihofer A, et al. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies novel methylation loci. *bioRxiv Prepr*. 2019. doi:https://doi.org/10.1101/585109

23. Yang SY, Ahmed S, Satheesh S V, Matthews J. Genome - wide mapping and analysis of aryl hydrocarbon receptor (AHR)- and aryl hydrocarbon receptor repressor (AHRR)- binding sites in human breast cancer cells. *Arch Toxicol*. 2018;92(1):225-240. doi:10.1007/s00204-017-2022-x

24. Krzyzewska IM, Ensink JBM, Nawijn L, et al. Genetic variant in CACNA1C is associated with PTSD in traumatized police officers. *Eur J Hum Genet*. 2018;26(2):247-257. doi:10.1038/s41431-017-0059-1

25. Logue MW, Miller MW, Wolf EJ, et al. An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clin Epigenetics*. 2020;12(1):1-14. doi:10.1186/s13148-020-0820-0

26. Vincenzes KA. Comparison of Civilian Trauma and Combat Trauma. *VISTAS Online*. 2013;45:1-8.

27. Tuerk PW, Ph D, Grubaugh AL, et al. Diagnosis and Treatment of PTSD-Related Compulsive Checking Behaviors in Veterans of the Iraq War : The Influence of Military Context on the Expression of PTSD Symptoms. *Am J Psychiatry*. 2009;(166):762-767.

28. Hagenaars MA, Fisch I, Minnen A Van. The effect of trauma onset and frequency on PTSD-associated symptoms. *J Affect Disord*. 2011;132(1-2):192-199. doi:10.1016/j.jad.2011.02.017

29. Kessler RC, Aguilar-gaxiola S, Alonso J, et al. Trauma and PTSD in the WHO World Mental Health Surveys. *Eur J Psychotraumatol*. 2017;8(5):1353383. doi:10.1080/20008198.2017.1353383

30. Howie H, Rijal CM, Ressler KJ. A review of epigenetic contributions to post-traumatic stress disorder. *Dialogues Clin Neurosci*. 2019;21(4):417-428. doi:10.31887/DCNS.2019.21.4/kressler

31. Nawijn L, Krzyzewska IM, van Zuiden M, et al. Oxytocin receptor gene methylation in male and female PTSD patients and trauma-exposed controls. *Eur Neuropsychopharmacol*. 2019;29(1):147-155. doi:10.1016/j.euroneuro.2018.10.006

32. Galanter JM, Gignoux CR, Oh SS, et al. Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. *Elife*. 2017;6:e20532. doi:10.7554/eLife.20532

33. Yuan V, Price EM, Del Gobbo G, et al. Accurate ethnicity prediction from placental DNA methylation data. *Epigenetics and Chromatin*. 2019;12(1):1-14. doi:10.1186/s13072-019-0296-3

34. Merikangas AK, Almasy L. Using the tools of genetic epidemiology to understand sex differences in neuropsychiatric disorders. *Genes, Brain Behav*. 2020;19:e12660. doi:10.1111/gbb.12660

35. Sartor C, McCutcheon V, Pommer N, et al. Common genetic and environmental contributions to posttraumatic stress disorder and alcohol dependence in young women. *Psychol Med*. 2011;41(7):1497-1505. doi:10.1017/S0033291710002072.Common

36. Duncan L, Ratanatharathorn A, Aiello AE, et al. Largest GWAS of PTSD (N = 20 070) yields genetic overlap with schizophrenia and sex differences in heritability. *Mol Psychiatry*. 2018;23:666-673. doi:10.1038/mp.2017.77

37. Goldstein JM, Jerram M, Abbs B, Whitfield-gabrieli S, Makris N. Sex Differences in Stress Response Circuitry Activation Dependent on Female Hormonal Cycle. *J Neurosci*. 2010;30(2):431-438. doi:10.1523/JNEUROSCI.3021-09.2010

38. Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress : a review. *Biol Psychol*. 2005;69:113-132. doi:10.1016/j.biopsycho.2004.11.009

39. Ramikie TS, Ressler KJ. Stress-related disorders, pituitary adenylate cyclase-activating peptide (PACAP)ergic system, and sex differences. *Dialogues Clin Neurosci*. 2016;18(4):403-413.

40. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296

41. Sheehan D, Yves L, Sheehan K, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I): The development and validation of a structured diagnostic

psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59(20):34-57.

42. Bernstein D, Fink L. *Childhood Trauma Questionnaire: A Retrospective Self-Report Manual*. San Antonio: The Psychological Corporation; 1998.

43. Jewkes R, Nduna M, Levin J, et al. A cluster randomized-controlled trial to determine the effectiveness of Stepping Stones in preventing HIV infections and promoting safer sexual behaviour amongst youth in the rural Eastern Cape, South Africa: Trial design, methods and baseline findings. *Trop Med Int Heal*. 2006;11(1):3-16. doi:10.1111/j.1365-3156.2005.01530.x

44. Weathers F, Blake D, Schnurr P, Kaloupek D, Marx B, Keane T. *The Life Events Checklist for DSM-5 (LEC-5)*. USA: National Center for PTSD; 2013.

45. Davidson JRT, Book SW, Colket JT, et al. Assessment of a new self-rating scale for post-traumatic stress disorder. *Psychol Med*. 1997;27(1):153-160. doi:10.1017/S0033291796004229

46. Saunders J, Aasland O, Babor T, De La Fuente J, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction*. 1993;88(6):791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

47. Radloff L. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas*. 1977;1(3):385-401. doi:10.1002/ardp.19552881111

48. Illumina. Infinium® MethylationEPIC BeadChip. *Illumina Mark*. 2015:4. https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/humanmethylationepic-data-sheet-1070-2015-008.pdf.

49. Min JL, Hemani G, Davey Smith G, Relton C, Suderman M. Meffil: efficient normalization and analysis of very large DNA methylation datasets. *Bioinformatics*. 2018;34(23):3983-3989. doi:10.1093/bioinformatics/bty476

50. R Core Team. R: A language and environment for statistical computing. 2018. https://www.r-project.org/.

51. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling.

Genome Biol. 2016;17(1):1-17. doi:10.1186/s13059-016-1066-1

52. Bibikova M, Barnes B, Tsan C, et al. Genomics High density DNA methylation array with single CpG site resolution Unmethylated locus Methylated locus Unmethylated locus Methylated locus. *Genomics*. 2011;98(4):288-295. doi:10.1016/j.ygeno.2011.07.007

53. Fortin J, Labbe A, Lemire M, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol*. 2014;15(503):1-17.

54. Leek JT, Storey JD. Capturing Heterogeneity in Gene Expression Studies by Surrogate Variable Analysis. *PLoS Genet*. 2007;3(9):e161. doi:10.1371/journal.pgen.0030161

55. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13(1). doi:10.1186/1471-2105-13-86

56. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. *R Stat Soceity*. 1995;57(1):289-300.

57. Suderman M, Staley JR, French R, Arathimos R, Simpkin A, Tilling K. Dmrff: Identifying Differentially Methylated Regions Efficiently With Power and Control. *bioRxiv*. 2018:508556. doi:10.1101/508556

58. Hannon E, Lunnon K, Schalkwyk L, Mill J. Interindividual methylomic variation across blood, cortex, and cerebellum: Implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics*. 2015;10(11):1024-1032. doi:10.1080/15592294.2015.1100786

59. Staley J, Battram T, Hemani G, Suderman M, Relton C, Guant T. The MRC-IEU catalog of epigenome-wide association studies. EWAS Catalog. http://ewascatalog.org/. Published 2017.

60. Li M, Zou D, Li Z, et al. EWAS Atlas : a curated knowledgebase of epigenome-wide association studies. *Nucleic Acids Res*. 2019;47(October 2018):983-988. doi:10.1093/nar/gky1027

61. Ressler K, Mercer K, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and PAC1 receptor. *Nature*. 2011;470(7335):492-497. doi:10.1097/CCM.0b013e31823da96d.Hydrogen

62. Almli LM, Mercer KB, Kerley K, et al. ADCYAP1R1 genotype associates with post-

traumatic stress symptoms in highly traumatized African-American females. *Am J Med Genet Part B Neuropsychiatr Genet*. 2013;162(3):262-272. doi:10.1002/ajmg.b.32145

63. Stevens JS, Almli LM, Fani N, et al. PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. *Proc Natl Acad Sci U S A*. 2014;111(8):3158-3163. doi:10.1073/pnas.1318954111

64. Schechter DS, Moser DA, Pointet VC, et al. The association of serotonin receptor 3A methylation with maternal violence exposure, neural activity, and child aggression. *Behav Brain Res*. 2017;325:268-277. doi:10.1016/j.bbr.2016.10.009

65. Tamir T, Bowman B, Agajanian M, et al. Gain-of-function genetic screen of the kinome reveals BRSK2 as an inhibitor of the NRF2 transcription factor. *J Cell Sci*. 2020;133:jcs241356. doi:10.13861/j.cnki.sece.2019.05.003

66. Kalsi G, Euesden J, Coleman JRI, et al. Genome-Wide Association of Heroin Dependence in Han Chinese. 2016:1-18. doi:10.1371/journal.pone.0167388

67. Kim JSM, Lilley BN, Zhang C, Shokat KM, Sanes JR, Zhen M. A chemical-genetic strategy reveals distinct temporal requirements for SAD-1 kinase in neuronal polarization and synapse formation. *Neural Dev*. 2008;3(23):1-14. doi:10.1186/1749-8104-3-23

68. Kishi M, Pan YA, Crump JG, Sanes JR. Mammalian SAD kinases are required for neuronal polarization. *Science (80-)*. 2005;307(5711):929-932. doi:10.1126/science.1107403

69. Sample V, Ramamurthy S, Gorshkov K, Ronnett G V, Chernoff J. Polarized activities of AMPK and BRSK in primary hippocampal neurons. 2015;26:1935-1946. doi:10.1091/mbc.E14-02-0764

70. Inoue E, Mochida S, Takagi H, et al. SAD: A Presynaptic Kinase Associated with Synaptic Vesicles and the Active Zone Cytomatrix that Regulates Neurotransmitter Release. *Neuron*. 2006;50(2):261-275. doi:10.1016/j.neuron.2006.03.018

71. Hiatt S, Thompson M, Prokop J, et al. Deleterious variation in BRSK2 associates with neurodevelopmental disorder. *Am J Hum Genet*. 2019;104(4):701-708. doi:10.1016/j.ajhg.2019.02.002

72. Feliciano P, Zhou X, Astrovskaya I, et al. Exome sequencing of 457 autism families recruited online provides evidence for novel ASD genes. *mpj Genomic Med*.

2019;4(19).

73. Ross D, Arbuckle M, Travis M, Dwyer J, van Schalkwyk G, Ressler K. An Integrated Neuroscience Perspective on Formulation and Treatment Planning for Posttraumatic Stress Disorder An Educational Review. *JAMA Psychiatry*. 2017;74(4):407-415. doi:10.1001/jamapsychiatry.2016.3325.An

74. van Marle H. PTSD as a memory disorder. *Eur J Psychotraumatol*. 2015;6:27633. doi:10.3402/ejpt.v6.27633

75. Krystal J, Neumeister A. Noradrenergic and serotonergic mechanisms in the neurobiology of posttraumatic stress disorder and resilience. *Brain Res*. 2009;1293:13-23. doi:10.1016/j.brainres.2009.03.044.Noradrenergic

76. Sherin JE, Nemeroff CB. Post-traumatic stress disorder: the neurobiological impact of psychological trauma. *Dialogues Clin Neurosci*. 2011;13:263-278.

77. Bright NJ, Carling D, Thornton C. Investigating the Regulation of Brain-specific Kinases 1 and 2 by Phosphorylation *. 2008;283(22):14946-14954. doi:10.1074/jbc.M710381200

78. Chen X, Gu X, Saiyin H, et al. Brain-selective Kinase 2 (BRSK2) Phosphorylation on PCTAIRE1 Negatively Regulates Glucose-stimulated Insulin Secretion in Pancreatic □ -Cells * □. 2012;287(36):30368-30375. doi:10.1074/jbc.M112.375618

79. Gastoł J, Kapusta P, Polus A, et al. Epigenetic mechanism in search for the pathomechanism of diabetic neuropathy development in diabetes mellitus type 1 (T1DM). *Endocrine*. 2020;68(1):235-240. doi:10.1007/s12020-019-02172-9

80. Jjingo D, Conley AB, Yi S V., Lunyak V V., King Jordan I. On the presence and role of human gene-body DNA methylation. *Oncotarget*. 2012;3(4):462-474. doi:10.18632/oncotarget.497

81. Edmondson D, Kronish IM, Shaffer JA, Falzon L, Burg MM. Posttraumatic stress disorder and risk for coronary heart disease: A meta-analytic review. *Am Heart J*. 2013;166(5):806-814. doi:10.1016/j.ahj.2013.07.031

82. Ryder AL, Azcarate PM, Cohen BE. PTSD and Physical Health. *Curr Psychiatry Rep*. 2018;20(12). doi:10.1007/s11920-018-0977-9

83. Mustafa T. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP). *Adv Pharmacol*. 2013;68:445-457. doi:10.1016/B978-0-12-411512-5.00021-X

84. Hammack SE, Cheung J, Rhodes KM, et al. Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): Roles for PACAP in anxiety-like behavior. *Psychoneuroendocrinology*. 2009;34(6):833-843. doi:10.1016/j.psyneuen.2008.12.013

85. Rudecki AP, Gray SL. PACAP in the Defense of Energy Homeostasis. *Trends Endocrinol Metab*. 2016;27(9):620-632. doi:10.1016/j.tem.2016.04.008

86. Mcmahon FJ, Akula N, Schulze TG, et al. Meta-analysis of genome-wide association data detects a risk locus for major mood disorders on chromosome 3p21.1. *Genome*. 2010;42(2):128-131. doi:10.1038/ng.523.Meta-analysis

87. Myers AJ, Gibbs JR, Webster JA, et al. A survey of genetic human cortical gene expression. *Nat Genet*. 2007;39(12):1494-1499. doi:10.1038/ng.2007.16

88. Mercer KB, Dias B, Shafer D, et al. Functional evaluation of a PTSD-associated genetic variant : estradiol regulation and ADCYAP1R1. 2016;(August):1-7. doi:10.1038/tp.2016.241

89. Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB. Posttraumatic Stress Disorder in the National Comorbidity Survey. *Arch Gen Psychiatry*. 1995;52(12):1048-1060. doi:10.1001/archpsyc.1995.03950240066012

90. Klose RJ, Bird AP. Genomic DNA methylation: The mark and its mediators. *Trends Biochem Sci*. 2006;31(2):89-97. doi:10.1016/j.tibs.2005.12.008

91. Maunakea AK, Nagarajan RP, Bilenky M, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature*. 2010;466(7303):253-257. doi:10.1038/nature09165

92. Anastasiadi D, Esteve-Codina A, Piferrer F. Consistent inverse correlation between DNA methylation of the first intron and gene expression across tissues and species. *Epigenetics and Chromatin*. 2018;11(1):1-17. doi:10.1186/s13072-018-0205-1

93. Dias BG, Ressler KJ. PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology*. 2013;38(1):245-247. doi:10.1038/npp.2012.147

94. Passos IC, Vasconcelos-Moreno MP, Costa LG, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *The Lancet Psychiatry*. 2015;2(11):1002-1012. doi:10.1016/S2215-0366(15)00309-0

95. Speer K, Upton D, Semple S, McKune A. Systemic low-grade inflammation in post-traumatic stress disorder: A systematic review. *J Inflamm Res*. 2018;11:111-121. doi:10.2147/JIR.S155903

96. Myint AM. Kynurenines: From the perspective of major psychiatric disorders. *FEBS J*. 2012;279(8):1375-1385. doi:10.1111/j.1742-4658.2012.08551.x

97. Wilson R, Wahl S, Pfeiffer L, et al. The dynamics of smoking-related disturbed methylation: A two time-point study of methylation change in smokers, non-smokers and former smokers. *BMC Genomics*. 2017;18(1):1-15. doi:10.1186/s12864-017-4198-0

98. Philibert R, Dogan M, Noel A, et al. Dose response and prediction characteristics of a methylation sensitive digital PCR assay for cigarette consumption in adults. *Front Genet*. 2018;9(APR):1-9. doi:10.3389/fgene.2018.00137

99. Joehanes R, Just AC, Marioni RE, et al. Epigenetic Signatures of Cigarette Smoking. *Circ Cardiovasc Genet*. 2016;9(5):436-447. doi:10.1161/CIRCGENETICS.116.001506

100. Ratanatharathorn A, Aiello A, Boks M, et al. Epigenetic Biomarkers Of PTSD: Updates From The EWAS Working Group of The PTSD PGC. *Eur Neuropsychopharmacol*. 2019;29:S750. doi:10.1016/j.euroneuro.2017.06.092

CHAPTER 5

FKBP5 INTRON 7 METHYLATION AND THE TRAJECTORY OF PTSD SYMPTOMS IN RAPE-EXPOSED WOMEN

ABSTRACT

Background: Emotional distress and posttraumatic stress disorder (PTSD) are often reported following rape. Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, a core regulator of the stress response, has been implicated in the aetiology and chronicity of PTSD. FK506 binding protein (FKBP5) is a co-chaperone and functional regulator of the glucocorticoid receptor. No studies to date have investigated longitudinal methylation changes in the *FKBP5* gene in rape-exposed women. **Aim:** The overarching aim was to investigate the relationship between *FKBP5* intron 7 methylation and PTSD symptoms over 6 months in a rape-exposed sample. We also investigated the interaction between childhood trauma, rs1360780 genotype and *FKBP5* methylation in relation to PTSD scores over time. The baseline (0-20 days), 3-month and 6-month post-rape data were used to investigate change over time. **Method:** Rape-exposed women (n = 852) were recruited from rape clinics in the Rape Impact Cohort Evaluation (RICE) umbrella study. PTSD symptom scores were derived from the Davidson Trauma Scale (DTS) at baseline, 3-months and 6-months post-rape. Methylation levels at five *FKBP5* intron 7 CpG sites (n = 96) were determined using EpiTYPER Sequenom MassArray technology. Genotyping of rs1360980 was completed using the Agena MassArray genotyping system. Mixed linear regression models were used to analyse the data. **Results:** Decreased *FKBP5* methylation was a predictor of increased PTSD symptoms scores at 3-months and 6-months post-rape. High childhood trauma and the CC genotype of rs1360780 resulted in decreased *FKBP5* methylation and increased PTSD scores at baseline. **Conclusion:** This is the first study to investigate longitudinal changes in *FKBP5* methylation in a relatively homogenous (in terms of demographic and trauma exposure) rape-exposed sample. The findings implicate *FKBP5* methylation in the aetiology and course of PTSD in the aftermath of rape and may guide future therapeutic interventions.

INTRODUCTION

The prevalence of posttraumatic stress disorder (PTSD) in sexual assault survivors is higher than those associated with other trauma types [1–4]. Women are at increased risk of developing PTSD compared to men and are also more frequently victims of sexual assault compared to men [2,5–12]. The hypothalamic-pituitary-adrenal (HPA) axis is integral to the regulation of the stress response and returning the body to homeostasis [13,14]. Dysfunction of the HPA-axis has been implicated in the aetiology and course of PTSD following trauma exposure [15–17]. A growing body of literature suggests that genetic and epigenetic mechanisms, such as DNA methylation, may mediate the interplay between trauma and HPA-axis functioning [18,19]. DNA methylation, which involves the addition of a methyl group to cytosine-guanine dinucleotides may mediate gene transcription by inhibiting binding of transcription factors of affected genes [20,21]. Decreased or increased expression of HPA-axis related genes in rape survivors may contribute to the phenotypic presentation of PTSD [18,22].

Most epigenetic studies of PTSD have focused on genes that are directly or indirectly associated with regulation of the HPA-axis [23,24]. One of the most commonly investigated genes is the FK506 binding protein gene *(FKBP5)* [23]. FKBP5 is a co-chaperone and important functional regulator of the glucocorticoid receptor (GR) [25,26]. Intracellularly, cortisol binds to a GR complex in the cytoplasm and enters the cell nucleus, where it binds to glucocorticoid response elements (GREs) [26,27]. Binding of the GR complex to GREs upregulates *FKBP5* transcription [26,27]. FKBP5, in turn, binds to GRs in the cytoplasm and inhibits GR translocation to the nucleus, thereby creating an ultra-short intracellular negative feedback loop [28,29]. Prolonged stress and increased expression of *FKBP5* may result in long-term methylation changes in HPA-axis genes and a dysregulated stress response. These changes may underlie the aetiology and trajectory of PTSD [23,24].

Single-nucleotide polymorphisms (SNPs) may also contribute to dysfunction of the HPA-axis by adversely mediating dynamic methylation/demethylation of *FKBP5* as part of the regulation of the ultra-short negative feedback loop [26]. Several *FKBP5* SNPs have been investigated in PTSD and childhood trauma studies, and many have delivered inconsistent results [19,30–32]. One SNP, rs1360780 (intron 2, 400 bp from a GRE), has delivered more consistent results [30–36]. The rs1360780 CT and TT genotypes have been linked to increased risk for developing PTSD [31,35], and increased *FKBP5* transcription, both in the absence and presence of GR binding to GREs [35]. Increased expression likely occurs as a result of the rs1360780 T allele and its adjacent alleles forming a TATA box, which often represents the transcription start site in promoters [37]. Transcription is initiated when RNA polymerase 2 (Pol II) and TATA

binding protein (TBP) binds to the TATA sequence [27,37]. Binding of TBP causes the DNA strand to bend at the binding site and a three-dimensional chromatin loop is formed, resulting in the GREs of intron 7 coming into direct contact with the GREs of intron 2 and PolII, thereby further enhancing transcription of *FKBP5* [35,37]. Increased transcription of *FKBP5* promotes cortisol resistance, delays activation of the negative feedback loop of the HPA-axis and prolongs the fight-or-flight response [27,37].

Childhood trauma is associated with an increased risk for PTSD [38–40] and a longer recovery period [3,41,42]. The rs1360780 SNP has been found to moderate the association between childhood trauma and PTSD since it is believed that prolonged increased circulating cortisol subsequent to childhood trauma may result in neuroendocrinological changes underlying the functioning of the HPA-axis, especially in the presence of at least one rs1360780 T allele [23,24,37,43,44]. These changes may include methylation/demethylation of CpG sites in the *FKBP5* gene [15–17].

Methylation studies have mainly focussed on an overlapping region in intron 7 of the *FKBP5* gene since it contains several glucocorticoid response elements (GREs) [35,45]. Studies investigating older participants (e.g. Korean Vietnam veterans and holocaust survivors) have found that increased methylation in intron 7 was associated with a history of trauma exposure [46], carrying the T allele or CT/TT genotype of rs1360780 [34] and with PTSD [34]. Studies investigating methylation and the interaction between childhood trauma and rs1360780 genotype in younger populations found that increased childhood trauma was associated with decreased methylation in T allele carriers in the offspring of holocaust survivors [46] and in German women of childbearing age [33]. Increased childhood trauma also interacted with the rs1360780 T allele to predict increased PTSD symptom severity in a predominantly African American, mixed gender, civilian sample [35]. Here, a 12.3% decrease in methylation in T allele carriers was observed, while the CC genotype carriers showed increased methylation in the same region [35].

Only one study has investigated longitudinal change in *FKBP5* intron 7 methylation in relation to change in PTSD symptom scores. The study investigated treatment responders and non-responders (Caucasian American veteran sample), following a nine-week mindfulness-based stress reduction intervention and found that non-responders showed decreased methylation compared to responders who showed increased methylation in the same region [47]. There were no significant differences in methylation levels between responders and non-responders at baseline, and baseline PTSD symptom severity scores were not associated with methylation levels [47].

No studies to date have investigated the relationship between *FKBP5* intron 7 methylation and PTSD symptom scores over time in rape-exposed women of African ethnicity. Longitudinal studies are needed to determine if *FKBP5* intron 7 methylation is associated with PTSD symptoms post-rape. In the present study, conducted in a South-African cohort of rape-exposed women in a low-income setting, our overarching aim was to investigate the relationship between longitudinal *FKBP5* intron 7 methylation and PTSD symptoms using baseline (0-20 days post-rape), 3-months and 6-months post-rape data. We also aimed to investigate the main effect of rs1360780 genotype and baseline childhood trauma scores on longitudinal change in PTSD symptoms and to investigate the interaction between childhood trauma, rs1360780 genotype and *FKBP5* methylation in relation to longitudinal change in PTSD scores.

METHODS

Design and setting

The study followed a longitudinal, prospective follow-up design. Samples and data were analysed from a subset of rape-exposed women (n = 96) recruited from the RICE study, a cohort study investigating the impact of rape on women's health and their use of health services in South Africa (n = 854). For a more detailed description of RICE's study methods, see Abrahams et al., 2017 [48]. Survivors of rape were recruited from four rape centres in and around the city of Durban located in the KwaZulu Natal province of South Africa. The rape centres provide comprehensive emergency care, including access to police, counselling, medical and forensic care. Rape-exposed women were informed of the study after presenting to and receiving treatment from the rape clinics. Interested participants were invited to the study site for enrolment.

Participants

All participants were women who had been raped within 20 days of the baseline visit. All participants were between 18 and 40 years of age and of Black African ethnicity. In the parent study, participants were excluded if they were severely distressed or in urgent need of psychiatric treatment/hospitalisation, if they had an intellectually disability or were more than 14 weeks pregnant at enrolment. Further exclusions were applied for this sub-study to control for factors that could influence methylation levels. Participants were excluded if they were pregnant or lactating (at any timepoint), if they had HIV seroconverted (at any timepoint) and if they met diagnostic criteria for PTSD as baseline due to a past traumatic event other than the

rape. Only participants with complete clinical datasets and blood samples with sufficient DNA at all timepoints were considered for inclusion.

Procedure and ethical considerations

Upon enrolment researchers explained the study procedure, obtained informed consent, and completed the baseline clinical interview and assessments with participants. Assessments included: a demographic questionnaire; self-report questionnaires measuring risk and protective factors for the development of PTSD; a rapid HIV antibody blood spot test; a human chorionic gonadotropin (hCG) urine pregnancy test; body mass index (BMI) assessment and; blood collection for DNA analysis. Nursing staff administered the HIV test, pregnancy tests, BMI assessment and blood collection. The nursing staff also recorded prescription medication use and smoking status at each timepoint. A research assistant administered the clinical interview (under supervision of a registered trauma counsellor or registered nurse) demographic questionnaire and self-report measures. Participants were invited back to the study site to complete a 3-month and 6-month follow-up visit where all assessments completed at baseline were repeated. The ninety-six participants were selected for the sub-study based on the quality of DNA that was available i.e. DNA concentration (>100 ng/µl) and purity (A_{260}/A_{280} ration > 1.8 and A_{260}/A_{230} ration > 2.0) of blood samples. Ethical approved to conduct RICE was obtained from the ethics committee at the South African Medical Research Council (SAMRC; EC019-10/2013) and approval to conduct the sub-study was obtained from the Health Research Ethics Committee at Stellenbosch University (S16/08/146).

Clinical measures

The Mini International Neuropsychiatric Interview

The Mini International Neuropsychiatric Interview (MINI) version 7.0.0 is a structured psychiatric interview that screens for 16 DSM-IV psychiatric disorders including mood disorders, anxiety disorders, alcohol and drug dependence, psychosis, eating disorders and personality disorders [49]. The MINI was used at baseline to screen for PTSD based on prior traumas other than the rape. The MINI has shown good reliability and validity in various settings [50,51].

Davidson Trauma Scale (DTS)

The Davidson trauma scale (DTS) is a self-report questionnaire used to assess seventeen symptoms of PTSD [52]. The DTS was administered at all timepoints and responses were measured on a 5-point Likert scale for symptom frequency ranging from 0 (‘not at all’) to 4

('every day') and symptom severity ranging from 0 ('not at all distressing') to 4 ('extremely distressing'). An uploading error resulted in missing value sets for the symptom severity subscale of the DTS which was corrected once it was identified. There were no missing values for the DTS frequency subscale. Missing DTS symptom severity values were imputed in the RICE study using a multiple imputation model while maintaining the multivariate normal distribution. The symptom frequency and severity scores were added together to produce a PTSD total score ranging between 0 and 136. A total score of 40 or more was considered indicative of PTSD. The DTS has shown excellent discriminating power for distinguishing between participants with and without PTSD at a cut-point of 40 [52,53]. The DTS showed excellent reliability in this study at each timepoint with a Cronbach alpha sore of .92 at baseline, .91 at 3-months and .93 at 6-months post-rape.

Childhood Trauma Questionnaire Short Form (CTQ-SF)

A modified version of the Childhood Trauma Questionnaire- Short Form (CTQ-SF) was used to measure exposure to childhood traumas before the age of 18 years, at the baseline visit [54,55]. The 14 items measuring childhood trauma centres around sexual abuse, physical abuse, emotional abuse, parental neglect and domestic violence. Responses were measured on a 4-point Likert scale ranging from 1 ('never') to 4 ('very often'). The modified CTQ-SF showed acceptable reliability in this study with a Cronbach alpha score of .75 at baseline.

Life Events Checklist (LEC)

A modified version of the LEC was used to measure lifetime exposure to different trauma types at the baseline visit [56,57]. The modified version of the LEC measures direct exposure to nine trauma types using a dichotomous 'yes/no' response. The trauma types measured were imprisonment, civil unrest/war, serious injury, being close to death, murder of a family member or friend, unnatural death of a family member or friend, murder of a stranger/s, robbed at gunpoint or knifepoint and kidnapped. The number of 'yes' responses were added together to yield a total score ranging from 0 to 9, indicating the cumulative lifetime trauma load of participants.

Alcohol Use Disorders Identification Test (AUDIT)

The Alcohol Use Disorders Identification Test-Consumption Scale (AUDIT-C) was used to measure hazardous alcohol use at baseline [58]. The original AUDIT consists of 10-items with responses measured on a 5-point Likert scale and response options specific to each individual item. A score of three or more on the AUDIT-C (the first three items of the AUDIT) is

considered indicative of hazardous drinking in women [59]. The AUDIT-C has shown good reliability and validity in various settings [60–66]. The AUDIT-C showed good reliability in this study with a Cronbach alpha score of .83.

Center for Epidemiologic Studies Depression Scale (CES-D)

The Center for Epidemiologic Studies Depression Scale (CES-D) was used to measure depression in accordance with DSM-IV criteria at baseline [67]. The CES-D is a twenty-item, self-report measure with responses measured on a 4-point Likert scale ranging from 0 ('rarely or none of the time') to 3 ('most or all of the time'). The CESD has shown good reliability and validity in various settings [68–77]. The CES-D showed good reliability in this study with a Cronbach alpha score of .89.

Experimental procedure

DNA was extracted from peripheral blood samples using the Gentra Puregene DNA extraction kit (Qiagen, Germany) and quantified by fluorimetry, using the Pico Green dsDNA quantitation reagent (ThermoFisher Scientific, Massachusetts, United States). Sample concentrations were normalised to 50 ng/ul and shipped on ice to Inqaba Biotec in Pretoria, South Africa for genotyping, busilfite conversion and methylation analysis.

Genotyping was completed using the Agena MassArray genotyping system (Agena Bioscience, California, United States). Polymerase chain reaction (PCR) was followed by single base extension using dideoxynucleoside terminators of the SNP specific primer. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was then used to identify SNP genotype.

Methylation analysis was completed using EpiTYPER Sequenom MassARRAY (Agena Bioscience, California, United States) technology. PCR primer design was completed using Sequenom Epidesigner software (www.epidesigner.com). The samples were randomly assigned to one of three plates i.e. 96 samples per plate, three plates to accommodate the samples collected at each timepoint. Samples were bisulfite-converted using the Zymo EZ DNA methylation kit (Zymo Research, California, United States). Bisulfite conversion was followed by PCR amplification of the target region using t7-promoter tagged primers (forward primer, 5'-aggaagagagTTGTTTTTGGAATTTAAGGTAATTGA-3' and reverse primer, 5'-cagtaatacgactcactatagggagaaggctCCTCCAACACTACTACTAAAAAAT AAAACA-3'). RNA transcription, base-specific RNA cleavage and finally DNA methylation quantification was completed using the SpectroCHIP array, MALDI-TOF mass spectrometry and EpiTYPER

software. Findings resulting from EpiTYPER technology has proven to be highly reproducible and has been found to detect methylation levels as low as 5% [78,79].

We followed the same approach as Klengel et al. (2013) in selecting the *FKBP5* intron 7 CpG sites to investigate methylation differences. Of the six CpG sites (divided into 3 bins) investigated in Klengel et al. (2013), we investigated two CpG sites in bin1 (Bin1-CpG1 and Bin1-CpG2) and two sites in bin 2 (Bin2-CpG1 and Bin2-CpG2-GRE). We could not investigate Bin2-CpG3 since the mass of silent peaks in the surrounding area precluded detection of methylation levels in this CpG site. We could also not investigate Bin3-CpG1-GRE since its mass was too low to be detected. We included an additional CpG site located 2bp from the end of the GRE in Bin3. We labelled the five CpG sites investigated in this study as CpG1-CpG5. Table1 presents the coordinates (chr6: 35558310-35558785) and sequences of the CpG sites investigated based on the Human Genome Build 37 (GRCh37/hg19).

Table 1: *FKBP5 intron 7 CpG sites investigated as predictors of PTSD score*

Coordinates	Sequence
35558310	GCTGTTTCTGGAATCCAAGGCAACTGACAAATTCTCTCTC
35558350	TTCTCTACTTGGAGAAGTATAAAAAAAAAATGGCTT**CG**
	CpG1
35558388	GGTTAGCTGCTTTCTTTCTTGTATCTCTGGTCACAGAGCC
35558428	TAGTGGCCCT**CG**AGGACTTGCAGTTGGGATAACAACTT
	CpG2
35558466	GGAGCCACAGTGCAGGCCTCTT**CG**TGACTCCTGTGAAG
	CpG3
35558504	GGTACAATC**CG**TTCAGCTCTGAAAAGCTGCACCCCACT
	CpG4
35558542	CCCCCAAGGAGCCACTTGGCAGAACGTGAACCTTTCTGT
35558581	CCTCAACCCAGGAAAAAAAAAGTACAAAAAGAACAAGT
35558619	CTAGGAACAAATAAGGGAACAAGTCTTGGATTCTACCCA
35558658	AAAAAGTTAAAAAAAAAAAAAAAAGCTGACACATAGGA
35558696	ACAAAATAAGAACACGGAGCTCCTT**CG**TTGTATATCAG
	CpG5
35558734	CTGTGCTATGTCAGTTGTTCTATTCTTCAGCAGCAGTGTT
35558774	GGAGGCAAGAGA

Underlined sections present glucocorticoid response elements. CpG sites in large bold font indicate the sites investigated in this study. Genomic coordinates are based on the Human Genome Build 37 (GRCh37/hg19).

Data analysis

Most variables did not conform to a normal distribution and non-parametric statistics were therefore used in the univariate analyses. Descriptive statistics were calculated for baseline demographic and clinical characteristics of the sample. Baseline age, HIV status, BMI score, smoking status, lifetime trauma exposure and medication use were investigated as potential confounding variables in relation to PTSD, methylation, rs1360780 genotype and childhood trauma. Depression and alcohol use were investigated as covariates of PTSD. Confounding/covarying variables were identified using Spearman's correlation coefficients, Chi square tests and Mann-Whitney U test statistics. The relationship between PTSD and rs1360780 genotype was investigated using Mann-Whitney U test statistics. An online calculator and Chi-squared test was used to determine if the observed rs1360780 genotype frequencies are consistent with Hardy-Weinberg equilibrium (http://www.dr-petrek.eu/documents/HWE.xls). Rs1360780 was grouped into CC genotype carriers and CT/TT genotype carriers given that the T allele and its adjacent alleles forms a TATA box which has a functional effect on gene expression [37]. The dichotomised rs1360780 variable (CC vs CT/TT) was used in all univariate and multivariate analyses.

Mixed linear regression models were used to investigate the main effect of methylation (baseline, 3-months and 6-months post-rape), rs1360780 genotype and childhood trauma on PTSD scores (baseline, 3-months and 6-months post-rape). Interaction effects between methylation, rs1360780 genotype and childhood trauma on PTSD scores were also investigated using mixed linear regression models. Childhood trauma scores were dichotomised using a median split to probe the significant interaction effects. Fisher's Z tests were used to compare the correlations between methylation and PTSD scores in the high childhood trauma & CC genotype vs high childhood trauma and CT/TT genotype groups as well as the low childhood trauma & CC genotype vs low childhood trauma and CT/TT genotype groups. Confounding/covarying variables significantly associated with PTSD, methylation, rs1360780 genotype and childhood trauma (resulting from the univariate analyses) were included in the models showing significant main and/or interaction effects.

RESULTS

Demographic and clinical characteristics of the sample

The baseline demographic characteristics of the sample for age, education, employment, relationship status, HIV status, smoking status, medication use, BMI, childhood trauma exposure and lifetime trauma exposure are presented in Table 2. The rs1360780 genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 1.87$, $p = .171$) and are also reported in Table 2.

Table 2: *Baseline demographic and clinical characteristics of the sample*

	n	%	*M*	*SD*	Range
Age	96	100	25.2	5.4	18-39
Basic education completed	96	100			
No	39	40.6			
Yes	57	59.4			
Employed	96	100			
No	74	77.1			
Yes	22	22.9			
Relationship status	96	100			
Single	20	20.8			
In a relationship	76	79.2			
HIV status	96	100			
Negative	50	52.1			
Positive	46	47.9			
Smoker	96	100			
No	84	87.5			
Yes	12	12.5			
Medication use	96	100			
No	64	66.7			
Yes	32	33.3			
BMI	96	100	25.9	6.1	18.0 – 47.5
Childhood trauma (CTQ-SF)	96	100	16.7	3.4	14-29
None	40	41.7			
Sexual abuse	21	21.9			
Physical abuse	37	38.5			
Emotional abuse	23	24.0			
Neglect	41	42.7			
Domestic violence	18	18.8			
Cumulative childhood trauma	96	100	1.5	1.5	0-5
Lifetime trauma (LEC)	96	100			
None	29	30.2			
Imprisonment	3	3.1			
Civil unrest or war	4	4.2			
Serious injury	11	11.5			
Close to death	27	28.1			
Murder of family or friend	16	16.7			
Unnatural death of family or friend	14	14.6			
Murder of a stranger	15	15.6			
Robbed at gun- or knifepoint	35	36.5			
Kidnapped	7	7.3			
Cumulative lifetime trauma	96	100	1.4	1.4	0-5
Alcohol use (AUDIT-C)	96	100	1.7	2.4	0-10
Not Hazardous	69	71.9			
Hazardous	27	28.1			
Depression (CES-D)	96	100	32.0	13.0	0-57
rs1360980 genotype	95	100			
CC	31	32.3			

CT	52	54.7
TT	12	12.6

Abbreviations:
Mean (M), standard deviation (SD), body mass index (BMI), Childhood Trauma Questionnaire Short Form (CTQ-SF), Life Events Checklist (LEC), Alcohol Use Disorders Identification Test Consumption subscale (AUDIT-C), Center for Epidemiologic Studies Depression Scale (CES-D).

Univariate analyses

The results of the univariate analyses are presented in Tables 3-5. Baseline depression (r = 0.50, p <.000) and baseline alcohol use (r = -0.20, p = .049) were correlated with PTSD. Baseline BMI score was correlated with CpG4 (r = 0.23, p = .033) and CpG5 (r = -0.24, p = .028) methylation. There was a significant difference in CpG5 methylation between participants who used prescription medication at baseline and those who did not (z = -2.03, p = .042). There were no differences between participants carrying the CC genotype and participant carrying the CT/TT genotype for any of the confounding/covarying variables. There was no significant difference in PTSD scores between CC and CT/TT genotype carriers.

Table 3: *Baseline confounding/covarying variables related to PTSD symptom scores, FKBP5 methylation and childhood trauma*

	Age		HIV status (negative vs positive)		BMI		Smoker (no vs yes)		Lifetime trauma		Alcohol use		Depression		medication (no vs yes)	
	r	*p*	*z*	*p*	*r*	*p*	*z*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*z*	*p*
PTSD (baseline)	-.199	.052	-1.07	.284	.077	.455	-0.49	.626	.062	.550	-.067	.514	.500	.000*	-1.46	.144
PTSD (3-months)	-.118	.252	-0.14	.889	-.037	.720	0.00	1.00	.061	.552	-.047	.650	.059	.568	-1.08	.282
PTSD (6-months)	.110	.286	-0.14	.889	-.015	.884	-0.29	.773	.047	.646	-.202	.049*	.222	.029*	-0.51	.608
CpG1 (baseline)	-.064	.566	-0.38	.702	.049	.658	-0.53	.598	.071	.524	.145	.188	-.071	.521	-1.28	.200
CpG2 (baseline)	-.200	.068	-0.02	.982	.082	.458	-1.06	.290	.007	.947	.065	.557	-.086	.438	-1.18	.239
CpG3 (baseline)	-.103	.353	-1.08	.282	.017	.876	-0.80	.423	.008	.941	.005	.966	-.062	.575	-0.01	.989
CpG4 (baseline)	.020	.859	-0.24	.811	.231	.033*	-0.87	.384	-.087	.430	-.088	.423	-.007	.947	-0.44	.663
CpG5 (baseline)	-.202	.063	-0.77	.441	.047	.672	-1.35	.179	.028	.799	-.002	.984	-.073	.507	-0.65	.514
CpG1 (3-months)	-.044	.689	-1.76	.079	-.074	.498	-1.02	.308	.180	.098	-.011	.917	-.104	.341	-0.38	.706
CpG2 (3-months)	-.060	.578	-1.41	.158	.005	.963	-1.93	.053	.092	.397	.005	.964	.063	.561	-1.15	.252
CpG3 (3-months)	.032	.766	-0.35	.728	.018	.866	-0.86	.392	.051	.638	-.024	.823	-.161	.134	-0.77	.440
CpG4 (3-months)	-.109	.319	-1.54	.124	.006	.956	-0.37	.712	.169	.121	.031	.780	-.006	.957	-1.24	.214
CpG5 (3-months)	.024	.827	-1.60	.109	.085	.432	-0.38	.706	-.004	.973	-.120	.266	-.119	.271	-0.32	.753
CpG1 (6-months)	.060	.585	-0.68	.496	-.469	.125	-0.91	.362	-.072	.514	.070	.527	-.130	.238	-1.57	.118
CpG2 (6-months)	-.082	.461	-0.97	.331	-.095	.393	-0.07	.946	-.076	.494	-.086	.438	-.012	.881	-0.48	.630
CpG3 (6-months)	-.076	.493	-0.53	.594	-.122	.270	-0.80	.424	-.001	.995	-.011	.921	-.009	.935	-0.07	.942
CpG4 (6-months)	-.073	.513	-0.93	.350	.082	.459	-1.08	.280	.043	.699	-.056	.616	-.131	.238	-0.43	.664
CpG5 (6-months)	-.033	.768	-1.36	.174	-.239	.028*	-0.48	.628	.073	.511	.137	.213	-.198	.071	-2.03	.042*
Childhood trauma (baseline)	-.099	.338	-.156	.876	.005	.961	-1.08	.279	.316	.002*	.196	.056	.193	.060	-.400	.689

*$p < .05$

Abbreviations: body mass index (BMI), posttraumatic stress disorder (PTSD).

Table 4: *Univariate associations of baseline confounding/covarying variables with rs1360980 CC vs CT/TT genotype*

	rs1360980 CC n = 31		rs1360980 CT/TT n = 64		Comparison by genotype grouping		
	M	*SD*	*M*	*SD*	n	*z*	*p*
Age	25.29	6.10	25.23	5.13	95	-0.25	.802
BMI	35.46	4.93	25.64	6.59	95	-1.50	.134
Childhood trauma	17.00	3.86	16.56	3.24	95	-0.07	.941
Lifetime trauma	1.39	1.56	1.38	1.28	95	-0.41	.680
Alcohol use	1.61	2.36	1.73	2.39	95	-0.22	.827
Depression	33.32	14.57	31.23	12.25	95	-1.06	.289
	n	%	n	%	n	χ^2	*p*
HIV positive	13	41.94	32	50.00	95	0.55	.460
Smoker	4	12.90	8	12.50	95	0.00	.956
Uses medication	9	29.03	23	35.94	95	0.45	.504

Abbreviations: body mass index (BMI)

Table 5: *Univariate associations of PTSD symptom scores with rs1360980 CC vs CT/TT genotype*

	rs1360980 CC n = 31		rs1360980 CT/TT n = 64		Comparison by genotype grouping		
	M	*SD*	*M*	*SD*	n	*z*	*p*
PTSD score (baseline)	70.95	22.03	63.88	18.87	95	-1.90	.058
PTSD score (3-months)	34.65	27.86	36.08	27.65	95	-0.37	.712
PTSD score (6-months)	21.76	28.35	27.95	28.02	95	-1.18	.240

Abbreviations: posttraumatic stress disorder (PTSD)

Main effect of methylation on PTSD

The main effects of *FKBP5* methylation as a predictor of PTSD are presented in Table 5 (models 1A-5A). Decreased CpG1 methylation levels were associated with increased PTSD scores at 3-months β = -.59, *t*(-2.76), *p* = .007 and 6-months β = -.45, *t*(-2.58), *p* = .011 post-rape. Decreased CpG2 β = -.49, *t*(-2.19), *p* = .030 and CpG5 β = -.57, *t*(-2.62), *p* = .010 methylation levels were also associated with increased PTSD scores at 6-months post-rape.

Decreased CpG1 methylation remained a significant predictor of increased PTSD at 3-months post-rape when adding baseline depression and alcohol as covariates β = -0.68, *t*(-2.14), *p* = .035 (see Supplementary Table 1). CpG1 and CpG2 methylation were no longer significant predictors of PTSD at 6-months post-rape, but increased baseline depression was a significant predictor of increased PTSD scores (see Supplementary Tables 2 and 3). CpG5 methylation remained a significant predictor of PTSD at 6-months post-rape when controlling for baseline BMI and baseline medication use β = -0.82, *t*(-2.29), *p* = .025 (see Supplementary Table 4). Baseline medication use was also a significant predictor of PTSD β = -14.12, *t*(-2.05), *p* = .044. When adding depression and alcohol use as covariates, CpG5 methylation was no longer a significant predictor of PTSD (see Supplementary Table 5). Baseline depression and alcohol use were not significant predictors of PTSD, but baseline medication use remained a significant predictor β = -14.06, *t*(-2.07), *p* = .042).

Main effect of rs1360780 genotype on PTSD

Rs1360780 genotype was not a significant predictor of PTSD in any of the models. The main effect of rs1360780 genotype as a predictor of PTSD is presented in Table 5 (models 1A-5A).

Main effect of childhood trauma on PTSD

The main effect of childhood trauma as a predictor of PTSD is presented in Table 5 (models 1A-5A). Increased childhood trauma scores were associated with increased PTSD scores at

baseline in all models i.e. models containing CpG1 β = 1.79, t(2.76), p = .008; CpG2 β = 1.85, t(2.87), p = .006; CpG3 β = 2.18, t(3.37), p = .001; CpG4 β = 1.94, t(2.86), p = .007; and CpG5 β = 1.78, t(2.77), p = .007. Increased childhood trauma remained a significant predictor of increased PTSD at baseline when including baseline depression and alcohol as covariates in all models (see Supplementary Tables 6-10). Increased baseline depression was also a significant predictor of increased baseline PTSD scores in all models i.e. models containing CpG1 β = 0.67, t(4.52), p < .000; CpG2 β = 0.63, t(4.43), p < .000; CpG3 β = 0.68, t(4.67), p < .000; CpG4 β = 0.69, t(4.77), p < .000; and CpG5 β = 0.68 t(4.62), p <.000 methylation. Decreased alcohol consumption at baseline was associated with increased PTSD scores at baseline for the models that included CpG1 β = -1.68, t(-2.02), p = .047; CpG3 β = -1.70, t(-2.09), p = .040; and CpG4 β = -1.71, t(-2.11), p = .038.

Table 6: *Summary statistics of variables predicting PTSD symptom scores at baseline and over time using mixed linear regression models*

Model		ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
	***FKBP5* CpG1 methylation**						
1A	Baseline*CpG1	-0.14	0.16	-0.90	.371	-0.45	0.18
	3-months*CpG1	-0.59	0.21	-2.76	.007*	-1.02	-0.17
	6-months*CpG1	-0.45	0.18	-2.58	.011*	-0.80	-0.11
	Baseline*genotype	-3.12	4.43	-0.70	.486	-12.07	5.84
	3-months*genotype	6.76	6.29	1.07	.288	-5.87	19.39
	6-months*genotype	7.08	5.59	1.27	.209	-4.03	18.19
	Baseline*ChildT	1.79	0.65	2.76	.008*	0.49	3.09
	3-months*ChildT	1.44	0.83	1.72	.090	-0.23	3.10
	6-months*ChildT	0.04	0.64	0.06	.956	-1.24	1.31
1B	Baseline*CpG1*rs1360780	-0.02	0.04	-0.51	.609	-0.10	0.06
	3-months*CpG1*rs1360780	-0.02	0.06	-0.39	.700	-0.13	0.09
	6-months*CpG1*rs1360780	0.01	0.05	0.16	.875	-0.09	0.10
	Baseline*ChildT	2.57	0.55	4.68	.000007*	1.48	3.65
	3-months*ChildT	0.76	0.64	1.19	.235	-0.50	2.02
	6-months*ChildT	-0.11	0.55	-0.19	.848	-1.19	0.97
1C	Baseline*genotype	-1.54	4.52	-0.34	.734	-10.52	7.44
	3-months*genotype	0.93	5.89	0.16	.874	-10.75	12.62
	6-months*genotype	0.64	6.21	0.10	.918	-11.70	12.98
	Baseline*CpG1*ChildT	0.02	0.01	3.07	.003*	0.01	0.03
	3-months*CpG1*ChildT	-0.00	0.01	-0.49	.623	-0.02	0.01
	6-months*CpG1*ChildT	-0.01	0.01	-1.29	.201	-0.02	0.01
1D	Baseline*CpG1	-0.11	0.15	-0.79	.433	-0.40	0.17
	3-months*CpG1	-0.55	0.16	-3.40	.001	-0.87	-0.23
	6-months*CpG1	-0.60	0.15	-3.98	.0001	-0.90	-0.30
	Baseline*rs1360780*ChildT	0.13	0.23	0.54	.591	-0.34	0.60
	3-months* rs1360780*ChildT	0.50	0.30	1.65	.104	-0.11	1.11
	6-months* rs1360780*ChildT	0.30	0.27	1.12	.267	-0.23	0.82
1E	Baseline*rs1360780*ChildT*CpG1	0.01	0.00	4.39	.00002*	0.00	0.01
	3-months* rs1360780*ChildT*CpG1	-0.00	0.00	-1.35	.180	-0.01	0.00
	6-months* rs1360780*ChildT*CpG1	-0.01	0.00	-3.29	.001*	-0.01	-0.00
	***FKBP5* CpG2 methylation**						
2A	Baseline*CpG2	-0.10	0.21	-0.50	.621	-0.52	0.31
	3-months*CpG2	-0.43	0.25	-1.74	.084	-0.92	0.06
	6-months*CpG2	-0.49	0.23	-2.19	.030*	-0.94	-0.05
	Baseline*genotype	-4.91	4.29	-1.15	.258	-13.54	3.73
	3-months*genotype	2.84	6.19	0.46	.647	-9.43	15.11
	6-months*genotype	5.75	5.38	1.07	.288	-4.93	16.43
	Baseline*ChildT	1.85	0.65	2.87	.006*	0.56	3.15
	3-months*ChildT	0.82	0.85	0.97	.336	-0.86	2.51
	6-months*ChildT	0.23	0.68	0.34	.736	-1.12	1.58

2B	Baseline*CpG2*rs1360780	-0.04	0.04	-0.93	.357	-0.13	0.05
	3-months*CpG2*rs1360780	-0.01	0.06	-0.12	.908	-0.14	0.12
	6-months*CpG2*rs1360780	0.01	0.06	0.15	.879	-0.10	0.12
	Baseline*ChildT	2.53	0.53	4.75	.000006*	1.48	3.59
	3-months*ChildT	0.46	0.63	0.73	.470	-0.80	1.71
	6-months*ChildT	-0.28	0.55	-0.51	.609	-1.36	0.80
2C	Baseline*genotype	-1.90	3.97	-0.48	.633	-9.79	5.99
	3-months*genotype	-0.42	5.45	-0.08	.938	-11.33	10.48
	6-months*genotype	0.34	4.81	0.07	.943	-9.19	9.88
	Baseline*CpG2*ChildT	0.02	0.01	3.66	.0004*	0.01	0.03
	3-months*CpG2*ChildT	-0.00	0.01	-0.11	.916	-0.02	0.01
	6-months*CpG2*ChildT	-0.01	0.01	-1.38	.169	-0.02	0.00
2D	Baseline*CpG2	-0.03	0.19	-0.17	.869	-0.41	0.35
	3-months*CpG2	-0.50	0.20	-2.48	.018	-0.91	-0.09
	6-months*CpG2	-0.61	0.19	-3.19	.002	-1.00	-0.23
	Baseline*rs1360780*ChildT	0.01	0.23	0.04	.972	-0.46	0.48
	3-months* rs1360780*ChildT	0.30	0.30	1.00	.326	-0.32	0.93
	6-months* rs1360780*ChildT	0.26	0.26	0.99	.327	-0.27	0.78
2E	Baseline*rs1360780*ChildT*CpG2	0.01	0.00	3.91	.0001*	0.00	0.01
	3-months* rs1360780*ChildT*CpG2	-0.00	0.00	-1.69	.094	-0.01	0.00
	6-months* rs1360780*ChildT*CpG2	-0.01	0.00	-3.70	.0002*	-0.01	-0.00
	***FKBP5* CpG3 methylation**						
3A	Baseline*CpG3	0.02	0.16	0.10	.924	-0.31	0.34
	3-months*CpG3	-0.10	0.21	-0.48	.630	-0.52	0.32
	6-months*CpG3	-0.26	0.22	-1.17	.244	-0.69	0.18
	Baseline*genotype	-2.21	4.20	-0.53	.600	-10.54	6.13
	3-months*genotype	1.20	6.28	0.19	.849	-11.34	13.74
	6-months*genotype	5.11	5.46	0.94	.351	-5.71	15.93
	Baseline*ChildT	2.18	0.65	3.37	.001*	0.90	3.46
	3-months*ChildT	0.65	0.84	0.77	.444	-1.03	2.33
	6-months*ChildT	0.47	0.75	0.63	.528	-1.01	1.95
3B	Baseline*CpG3*rs1360780	-0.02	0.04	-0.39	.698	-0.09	0.06
	3-months*CpG3*rs1360780	0.01	0.06	0.17	.866	-0.10	0.12
	6-months*CpG3*rs1360780	0.03	0.05	0.50	.617	-0.08	0.13
	Baseline*ChildT	2.60	0.53	4.91	.000002*	1.56	3.65
	3-months*ChildT	0.58	0.63	0.91	.362	-0.67	1.82
	6-months*ChildT	-0.17	0.56	-0.31	.759	-1.27	0.93
3C	Baseline*genotype	1.55	4.03	0.39	.701	-6.44	9.54
	3-months*genotype	-1.20	5.37	-0.22	.824	-11.91	9.51
	6-months*genotype	0.03	4.76	0.01	.995	-9.39	9.45
	Baseline*CpG3*ChildT	0.02	0.01	3.90	.0002*	0.01	0.03
	3-months*CpG3*ChildT	0.00	0.01	0.72	.477	-0.01	0.02
	6-months*CpG3*ChildT	-0.00	0.01	-0.61	.542	-0.01	0.01

3D	Baseline*CpG3	0.14	0.15	0.95	.350	-0.16	0.45
	3-months*CpG3	-0.21	0.17	-1.22	.230	-0.55	0.14
	6-months*CpG3	-0.35	0.17	-2.06	.044	-0.68	-0.01
	Baseline*rs1360780*ChildT	0.17	0.23	0.74	.467	-230	0.64
	3-months* rs1360780*ChildT	0.24	0.30	0.78	.439	-0.37	0.84
	6-months* rs1360780*ChildT	0.31	0.27	1.13	.264	-0.24	0.86
3E	Baseline*rs1360780*ChildT*CpG3	0.01	0.00	4.76	.000004*	0.01	0.01
	3-months* rs1360780*ChildT*CpG3	-0.00	0.00	-0.90	.368	-0.01	0.00
	6-months* rs1360780*ChildT*CpG3	-0.01	0.00	-2.86	.005*	-0.01	-0.00
	***FKBP5* CpG4 methylation**						
4A	Baseline*CpG4	-0.02	0.23	-0.09	.926	-0.49	0.45
	3-months*CpG4	-0.27	0.28	-0.99	.328	-0.84	0.29
	6-months*CpG4	-0.41	0.26	-1.59	.116	-0.93	0.10
	Baseline*genotype	-3.60	4.31	-0.84	.409	-12.38	5.18
	3-months*genotype	1.81	6.44	0.28	.780	-11.29	14.92
	6-months*genotype	6.77	5.44	1.24	.218	-4.11	17.65
	Baseline*ChildT	1.94	0.69	2.86	.007*	0.57	3.30
	3-months*ChildT	1.03	0.89	1.16	.252	-0.77	2.83
	6-months*ChildT	0.65	0.73	0.89	.378	-0.71	2.12
4B	Baseline*CpG4*rs1360780	-0.01	0.04	-0.30	.765	-0.10	0.07
	3-months*CpG4*rs1360780	-4.12	0.06	0.00	1.00	-0.11	0.11
	6-months*CpG4*rs1360780	0.03	0.05	0.52	.604	-0.07	0.13
	Baseline*ChildT	2.64	0.55	4.81	.000004*	1.56	3.73
	3-months*ChildT	0.78	0.65	1.20	.232	-0.51	2.06
	6-months*ChildT	-0.10	0.57	-0.17	.863	-1.25	1.02
4C	Baseline*genotype	-0.05	4.04	-0.01	.990	-8.22	8.11
	3-months*genotype	-0.10	5.60	4.52	.866	-15.87	13.87
	6-months*genotype	2.39	4.83	0.50	.624	-7.46	12.25
	Baseline*CpG4*ChildT	0.02	0.01	4.20	.0002*	0.01	0.04
	3-months*CpG4*ChildT	0.01	0.01	0.95	.360	-0.01	0.02
	6-months*CpG4*ChildT	-0.00	0.01	-0.66	.521	-0.02	0.01
4D	Baseline*CpG4	0.03	0.22	0.13	.896	-0.40	0.46
	3-months*CpG4	-0.35	0.24	-1.47	.144	-0.82	0.12
	6-months*CpG4	-0.48	0.23	-2.11	.037*	-0.38	0.55
	Baseline*rs1360780*ChildT	0.09	0.23	0.37	.710	-0.38	0.55
	3-months* rs1360780*ChildT	0.31	0.30	1.01	.317	-0.30	0.91
	6-months* rs1360780*ChildT	0.37	0.27	1.36	.178	-0.17	0.90
4E	Baseline*rs1360780*ChildT*CpG4	0.01	0.00	4.64	.000007*	0.00	0.01
	3-months* rs1360780*ChildT*CpG4	-0.00	0.00	-0.68	.499	-0.01	0.00
	6-months* rs1360780*ChildT*CpG4	-0.00	0.00	-2.62	.010*	-0.01	0.00
	***FKBP5* CpG5 methylation**						
5A	Baseline*CpG5	0.07	0.20	0.37	.712	-0.32	0.46
	3-months*CpG5	-0.20	0.25	-0.81	.419	-0.70	0.30
	6-months*CpG5	-0.57	0.22	-2.62	.010*	-1.00	-0.14

	Baseline*genotype	-3.88	4.02	-0.97	.336	-11.84	4.08
	3-months*genotype	0.65	5.95	0.11	.914	-11.22	12.51
	6-months*genotype	7.55	5.23	1.44	.152	-2.81	17.90
	Baseline*ChildT	1.78	0.64	2.77	.007*	0.50	3.06
	3-months*ChildT	0.60	0.80	0.75	.458	-1.00	2.20
	6-months*ChildT	0.62	0.64	0.96	.341	-0.66	1.89
5B	Baseline*CpG5*rs1360780	-0.02	0.06	-0.40	.693	-0.14	0.10
	3-months*CpG5*rs1360780	0.02	0.08	0.23	.823	-0.14	0.17
	6-months*CpG5*rs1360780	-0.03	0.07	-0.41	.681	-0.17	0.11
	Baseline*ChildT	2.53	0.55	4.57	.00001*	1.43	3.62
	3-months*ChildT	0.47	0.62	0.77	.442	-0.74	1.69
	6-months*ChildT	0.13	0.55	0.24	.808	-0.94	1.21
5C	Baseline*genotype	0.61	3.69	0.17	.869	-6.69	7.91
	3-months*genotype	-1.85	5.32	-0.35	.729	-12.45	8.75
	6-months*genotype	3.75	4.75	0.79	.432	-5.66	13.16
	Baseline*CpG5*ChildT	0.03	0.01	3.87	.0002*	0.01	0.04
	3-months*CpG5*ChildT	0.00	0.01	0.32	.751	-0.02	0.02
	6-months*CpG5*ChildT	-0.02	0.01	-1.96	.051	-0.03	0.00
5D	Baseline*CpG5	0.20	0.17	1.17	.242	-0.14	0.53
	3-months*CpG5	-0.30	0.20	-1.53	.129	-0.68	0.09
	6-months*CpG5	-0.57	0.19	-2.99	.003*	-0.94	-0.19
	Baseline*rs1360780*ChildT	0.08	0.21	0.36	.716	-0.34	0.50
	3-months* rs1360780*ChildT	0.18	0.28	0.64	.526	-0.38	0.74
	6-months* rs1360780*ChildT	0.38	0.25	1.51	.153	-0.12	0.88
5E	Baseline*rs1360780*ChildT*CpG5	0.01	0.00	4.51	.00001*	0.01	0.02
	3-months* rs1360780*ChildT*CpG5	-0.00	0.00	-1.23	.219	-.01	0.00
	6-months* rs1360780*ChildT*CpG5	-0.01	0.00	-3.46	.001*	-0.01	0.00

*p < .05

Abbreviations: FK506 binding protein *(FKBP5)*, childhood trauma (ChildT)

Interaction between methylation and rs1360780 CC vs CT/TT genotype

The results of the interaction between methylation and rs1360780 genotype are presented in Table 5 (models 1B-5B). The interaction between methylation and rs1360780 genotype was not a significant predictor of PTSD in any of the models.

Interaction between methylation and childhood trauma

The results of the interaction between methylation and childhood trauma are presented in Table 5 (models 1C-5C). The interaction between childhood trauma and methylation was a significant predictor of PTSD at baseline for all CpG sites. However, when comparing the correlation between methylation and PTSD for the groups with low vs high childhood trauma, there were no significant differences (see Supplementary Table 11).

Interaction between childhood trauma and rs1360780 genotype

The results of the interaction between childhood trauma and rs1360780 genotype are presented in Table 5 (models 1D-5D). The interaction between childhood trauma and rs1360780 genotype was not a significant predictor of PTSD in any of the models.

Interaction between methylation, rs1360780 genotype and childhood trauma

The results of the interaction between methylation, rs1360780 genotype and childhood trauma are presented in Table 5 (models 1E-5E). The interaction between methylation, rs1360780 genotype and childhood trauma was a significant predictor of PTSD at baseline and 6-months post-rape in all models.

When comparing the correlations between methylation and PTSD for the groups with high childhood trauma and CC genotype vs high childhood trauma and CT/TT genotype, there was a significant difference between the correlations for CpG3 (*Fisher's z* = -8.70, $p < .000$) at baseline (see Supplementary Table 12). Increased baseline PTSD scores were associated with decreased methylation at CpG3 in participants carrying the CC genotype ($r = -0.58$, $p = .040$) but not in participants carrying the CT/TT genotype ($r = -.003$, $p = .989$). The interaction effect was no longer significant when adding depression and alcohol use as covariates (see Supplementary Table 13). Increased baseline depression was a significant predictor of increased PTSD scores at baseline $\beta = 0.72$ $t(4.87)$, $p < .000$.

There was a significant difference between the correlations for CpG5 methylation and PTSD at baseline (*Fisher's z* = -5.89, $p < .000$). Increased baseline PTSD scores were

associated with decreased methylation at CpG5 in participants carrying the CC genotype (r = -0.83, p < .001) but not in participants carrying the CT/TT genotype (r = .057, p = .774). The interaction effect was no longer significant when adding baseline BMI and medication use as control variables (see Supplementary Table 14) as well as baseline depression and alcohol use as covariates (see Supplementary Table 15). Increased baseline depression was a significant predictor of increased baseline PTSD scores β = 0.74 t(4.90), p < .000.

When comparing the correlations between methylation and PTSD for the groups with low childhood trauma and CC genotype vs low childhood trauma and CT/TT genotype, there was a significant difference between the correlations for CpG5 at 6-months post-rape (*Fisher's* z = 1.80, p = .036). However, the correlation in the group with low childhood trauma and CC genotype (r = 0.32, p = .271) was not significant, neither was the correlation in the group with low childhood trauma and CT/TT genotype (r = -0.31, p = .103).

DISCUSSION

The overarching aim of this study was to determine if longitudinal change in *FKBP5* intron 7 methylation was a significant independent predictor of longitudinal change in PTSD scores. We also investigated the relationship between childhood trauma, rs1360780 and PTSD and the interaction effect between methylation, childhood trauma and rs1360780 as predictors of PTSD.

Decreased CpG1 methylation was a significant independent predictor of increased PTSD at 3-months and 6 months post-rape, and decreased CpG2 and CpG5 methylation were significant independent predictors of PTSD at 6-months post-rape. These results relate to those from the only prior longitudinal study investigating FKBP5 intron 7 methylation [47]. They found that responders (>10 point reduction in PTSD symptom scores) to a mindfulness-based stress reduction intervention showed increased methylation in CpG4 from pre-intervention to post-intervention, compared to non-responders who showed decreased methylation over time [47]. Decreased methylation is likely to result in overexpression of *FKBP5* which may disrupt the ultra-short intracellular negative feedback loop regulated by the interaction between glucocorticoid receptor and FKBP5 [28,29]. Increased expression of *FKBP5* will likely result in reduced intracellular binding of cortisol to GRs (since FKBP5 inhibits GR functioning) and this may result in delayed activation of the negative feedback loop of the HPA-axis (binding of cortisol to GRs signals the activation of the negative feedback loop) and a prolonged stress

response [27,30,35,37,80]. Prolonged activation of the stress response may underlie or exaggerate PTSD symptoms, especially symptoms related to hypervigilance [14,81].

The rs1360780 genotype (CC vs CT/TT), either independently or in interaction with childhood trauma and methylation, was not a significant independent predictor of PTSD. This finding is inconsistent with prior cross-sectional studies where the T allele of rs1360780 was found to interact with increased childhood trauma in predicting increased PTSD symptom severity [30,31,35]. The T allele of rs1360980 is generally thought to have a functional effect on *FKBP5* expression by upregulating *FKBP5* transcription through the formation of a TATA box and altered chromatin structure [27,35,37]. However, the aforementioned studies did not take the effect of *FKBP5* methylation into account [31,35,82]. While the T allele of rs1360780 is likely to result in increased expression of *FKBP5*, decreased *FKBP5* promoter methylation may have the same effect [18,23,44]. The functional effect of methylation may be stronger than that of the rs1360780 genotype and may overrule the effect of the T allele on *FKBP5* expression, although rs1360780 may also contribute to decreased methylation in intronic regions [83,84]. The sample characteristics of prior studies are also different to the characteristics of our sample since one prior study investigated a North American populations with a mixture of trauma types [35] and another investigated an older veteran sample [31]. Characteristics such as gender, ethnicity and trauma type may have an effect on methylation and on the risk of developing PTSD and limits the comparison results between studies [85–87].

Increased childhood trauma was an independent predictor of increased PTSD scores at baseline. Childhood trauma is a well-established risk factor for the development of PTSD following adulthood revictimization [38–40]. Prolonged increased circulating cortisol subsequent to childhood trauma may result in neuroendocrinological changes underlying the functioning of the HPA-axis [88,89]. These neuroendocrinological changes may be more pronounced when childhood trauma occurs at developmentally sensitive periods and may include methylation/demethylation of *FKBP5* CpG sites [35,37]. In fact, an interaction effect between childhood trauma and methylation at each CpG sites was observed in this study but the effect was not significant upon further investigation of the correlations in the childhood trauma vs genotype subgroups. It is plausible that an incremental increase in childhood trauma results in an incremental decrease in *FKBP5* methylation as well as increase in baseline PTSD scores, and that this effect may be masked by categorising childhood trauma exposure into low and high groups [90]. Childhood trauma also has adverse effects on emotional development and may result in maladaptive coping, social and emotional skills, which may increase the risk for developing PTSD following rape, independent of the effects of methylation [91,92].

When considering the interaction between methylation, rs1360780 genotype and childhood trauma, we found that increased baseline PTSD scores were associated with decreased methylation at CpG3 and CpG5 in participants with high childhood trauma who possessed the CC genotype. Conflicting findings related to the association between methylation, rs1360780 genotype and childhood trauma have been reported in prior cross-sectional studies [34,35,46]. Some studies report that T allele carriers with increased childhood trauma show decreased *FKBP5* intron 7 methylation at CpG1 [33], CpG3 and CpG4 [35], while others found that increased childhood trauma and carrying the T allele is associated with increased methylation in and around CpG4 [34,46]. There are currently no longitudinal studies investigating both rs1360780 genotype and *FKBP5* intron 7 methylation in relation to PTSD and the majority of cross-sectional studies have investigated *FKBP5* methylation levels in relation to PTSD, irrespective of the time since the traumatic event occurred [34,35,46]. This limits the comparison of our findings with previous findings since we measured methylation change in the immediate aftermath of trauma. Our study is also the first investigating participants of African ethnicity and rs1360780; therefore it is important to note that the SNP may have an ethnicity-dependent functional effect [85,86].

The independent effects observed for decreased CpG1, CpG2 and CpG5 methylation in relation to PTSD symptom severity at 6-months post-rape, as well as the interaction effects observed for decreased methylation at CpG3 and CpG5 in participants with high childhood trauma and carrying the CC genotype, were no longer significant when adding baseline depression as a covariate to the model. Decreased CpG1 methylation as an independent predictor of increased PTSD at 3-months post-rape survived the addition of depression as a covariate. Although depression was a strong predictor of PTSD and reduced the effect of methylation on PTSD symptom severity in most models, the role of depression in the development of PTSD must be considered carefully [93]. Depression and PTSD comorbidity is highly prevalent in survivors of interpersonal trauma [94,95]. They are considered distinct disorders, but they do also share overlapping symptom components; most notably, both disorders are associated with negative affect [93]. A study investigating survivors of sexual assault reported that PTSD symptom severity decreased in proportion to depression symptom severity over time, and that depression scores were fully mediated by PTSD scores [4]. Depression may explain a significant proportion of variance in PTSD simply based on overlapping symptom constructs, in which case it cannot be considered an independent variable but rather a variable closely related to the dependent variable [93–95].

Increased baseline alcohol consumption was added to the models along with baseline depression scores, and higher alcohol consumption was associated with decreased PTSD symptoms at baseline. Although the finding seems counterintuitive, it is not completely unsupported [96,97]. A retrospective study investigating male and female veterans found that female veterans reporting moderate alcohol consumption showed a decreased risk for PTSD compared to those who abstained, light drinkers and hazardous drinkers [96]. No difference in risk for PTSD was observed between light and moderate drinkers in their male subsample [96]. Another study found that moderate drinkers showed decreased PTSD scores following admission to an emergency department for a traumatic injury and at 3-months post-admission [97]. Although further investigation of the relationship between alcohol consumption and change in PTSD scores over time is needed, it is plausible that moderate intake of alcohol may protect against fear conditioning related to traumatic memories or may provide temporary relief from distressing symptoms [97,98].

The findings reported in this study should be interpreted in light of some limitations. First, our sample size was small, thus the study was statistically underpowered. Second, although substantial evidence exists linking PTSD to *FKBP5* genotype and differential methylation, PTSD remains a complex disease and its aetiology and trajectory is likely due to an interaction between many genes and biological pathways. Third, whole blood was used to investigate differential methylation while differential methylation in brain tissue is more likely to contribute to PTSD pathophysiology. However, *FKBP5* intron 7 methylation levels in whole blood have been found to correlate with methylation levels in brain tissue in animal studies [99,100]. Blood is also easily accessible and blood biomarkers of PTSD risk could be used to prioritise treatment for individuals most likely to develop PTSD following trauma exposure. Fourth, we could not compare post-rape epigenetic changes to pre-rape epigenetic markers since we did not have pre-rape data i.e. the baseline assessment was completed within 20 days following the rape. This is a limitation for determining whether a cause-effect relationship exists between rape exposure and epigenetic changes. Finally, we did not use laboratory methods to identify and count specific blood cell types in individual samples. Potential confounding in methylation findings, due to cell-type composition and change in blood cell-type compositions over time, cannot be determined.

Despite these limitations, there are notable strengths. The rs1360980 genotype and the CpG sites in and around GREs investigated in this study have been associated with functional effects. Investigating these sites thus contributes to the understanding of PTSD aetiology. In

addition, we investigated a relatively homogenous sample, both in terms of demographic characteristics (young women from low socio-economic position and from the same ethnicity group) and trauma exposure, enabling us to control various confounding factors.

In summary, the study provides evidence linking *FKBP5* methylation to PTSD symptoms scores at 3-months and 6-months post-rape. Childhood trauma and *FKBP5* rs1360780 CC genotype influenced the relationship between *FKBP5* methylation and PTSD at baseline. This is the second study to investigate longitudinal changes in methylation levels in intron 7 of the *FKBP5* gene, but the first to investigate longitudinal changes in a rape-exposed, African sample. Rape carries a high conditional risk for PTSD and identifying the underlying molecular mechanisms contributing to the aetiology and course of PTSD in the aftermath of rape may guide future therapeutic interventions.

REFERENCES

1. Elklit A, Christiansen DM. ASD and PTSD in rape victims. *J Interpers Violence*. 2010;25(8):1470-1488. doi:10.1177/0886260509354587

2. Elklit A, Christiansen DM. Risk factors for posttraumatic stress disorder in female help-seeking victims of sexual assault. *Violence Vict*. 2013;28(3):552-568. doi:10.1891/0886-6708.09-135

3. Möller AT, Bäckström T, Söndergaard HP, Helström L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One*. 2014;9(10):1-9. doi:10.1371/journal.pone.0111136

4. Nickerson A, Steenkamp M, Aerka IM, et al. Prospective investigation of mental health following sexual assault. *Depress Anxiety*. 2013;30(5):444-450. doi:10.1002/da.22023

5. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

6. Moor A, Farchi M. Is rape-related self blame distinct from other post traumatic attributions of blame? A comparison of severity and implications for treatment. *Women Ther*. 2011;34(4):447-460. doi:10.1080/02703149.2011.591671

7. Breslau N. The epidemiology of posttraumatic stress disorder: What is the extent of the problem? *J Clin Psychiatry*. 2001;62(17):16-22.

8. Hetzel-Riggin MD, Roby RP. Trauma Type and Gender Effects on PTSD, General Distress, and Peritraumatic Dissociation. *J Loss Trauma*. 2013;18(1):41-53. doi:10.1080/15325024.2012.679119

9. Kolltveit S, Lange-Nielsen II, Thabet AAM, et al. Risk factors for PTSD, anxiety, and depression among adolescents in Gaza. *J Trauma Stress*. 2012;25(2):164-170. doi:10.1002/jts.21680

10. Meewisse M-L, Reitsma JB, De Vries G-J, Gersons BPR, Olff M. Cortisol and post-traumatic stress disorder in adults. *Br J Psychiatry*. 2007;191(5):387-392. doi:10.1192/bjp.bp.106.024877

11. Stein MB, Kennedy C. Major depressive and post-traumatic stress disorder comorbidity in female victims of intimate partner violence. *J Affect Disord*. 2001;66(2-3):133-138. doi:10.1016/S0165-0327(00)00301-3

12. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: A quantitative review of 25 years of research. *Psychol Bull*. 2006;132(6):959-992. doi:10.1037/0033-2909.132.6.959

13. Gaete HP. Hypothalamus-pituitary-adrenal (HPA) axis , chronic stress , hair cortisol , metabolic syndrome and mindfulness. *Integr Mol Med*. 2016;3(5):776-779. doi:10.15761/IMM.1000244

14. Daskalakis NP, Mcgill MA, Lehrner A, Yehuda R. Endocrine aspects of PTSD: hypothalamic-pituitary-adrenal (HPA) axis and beyond. In: Martin C, Preedy V, Patel V, eds. *Comprehensive Guide to Post-Traumatic Stress Disorders*. Switzerland: Springer International Publishing; 2015. doi:10.1007/978-3-319-08359-9

15. Faravelli C, Sauro C Lo, Godini L, et al. Childhood stressful events, HPA axis and anxiety disorders. *World J Psychiatry*. 2012;2(1):13-25. doi:10.5498/wjp.v2.i1.13

16. Hulme PA. Childhood Sexual Abuse , HPA Axis Regulation , and Mental Health : An Integrative Review. *West J Nurs Res*. 2011;33(8):1069-1097. doi:10.1177/0193945910388949

17. Klaassens ER, Giltay EJ, Cuijpers P, van Veen T, Zitman FG. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: A meta-analysis. *Psychoneuroendocrinology*. 2012;37(3):317-331. doi:10.1016/j.psyneuen.2011.07.003

18. Zannas AS, Provençal N, Binder EB. Review Epigenetics of Posttraumatic Stress Disorder : Current Evidence , Challenges , and Future Directions. *Biol Psychiatry*. 2015;78(5):327-335. doi:10.1016/j.biopsych.2015.04.003

19. Mehta D, Binder EB. Neuropharmacology Gene x environment vulnerability factors for PTSD : The HPA-axis. *Neuropharmacology*. 2012;62:654-662. doi:10.1016/j.neuropharm.2011.03.009

20. Isles AR, Wilkinson LS. Epigenetics: What is it and why is it important to mental disease? *Br Med Bull*. 2008;85(1):35-45. doi:10.1093/bmb/ldn004

21. Kato T, Iwamoto K. Comprehensive DNA methylation and hydroxymethylation

analysis in the human brain and its implication in mental disorders. *Neuropharmacology*. 2014;80:133-139. doi:10.1016/j.neuropharm.2013.12.019

22. Klengel T, Pape J, Binder EB, Mehta D. The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology*. 2014;80:115-132. doi:10.1016/j.neuropharm.2014.01.013

23. Morrison FG, Miller MW, Logue MW, Assef M, Wolf EJ. DNA methylation correlates of PTSD: Recent findings and technical challenges. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;90(November 2018):223-234. doi:10.1016/j.pnpbp.2018.11.011

24. Vinkers CH, Kalafateli AL, Rutten BP, et al. Traumatic stress and human DNA methylation: A critical review. *Epigenomics*. 2015;7(4):593-608. doi:10.2217/epi.15.11

25. Pratt WB, Toft DO. Steroid Receptor Interactions with Heat Shock Protein and Immunophilin Chaperones *. *Endrocrine Rev*. 1997;18(3):306-360.

26. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*. 2009;34(SUPPL. 1):186-195. doi:10.1016/j.psyneuen.2009.05.021

27. Zannas AS, Wiechmann T, Gassen NC, Binder EB. Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications. *Neuropsychopharmacology*. 2016;41(1):261-274. doi:10.1038/npp.2015.235

28. Szyf M. How do environments talk to genes? *Nat Neurosci*. 2013;16(1):1-4.

29. Jaaskelainen T, Makkonen H, Palvimo J. Steroid up-regulation of FKBP51 and its role in hormone signaling. *Curr Opin Pharmacol*. 2011;11:326-331. doi:10.1016/j.coph.2011.04.006

30. Binder EB, Bradley RG, Epstein MP, et al. Association of FKBP5 Polymorphisms and Childhood Abuse With Risk of Posttraumatic Stress Disorder Symptoms in Adults. *JAMA*. 2008;299(11):1291-1305.

31. Watkins LE, Han S, Harpaz-rotem I, et al. Psychoneuroendocrinology FKBP5 polymorphisms , childhood abuse , and PTSD symptoms : Results from the National

Health and Resilience in Veterans Study. *Psychoneuroendocrinology*. 2016;69:98-105. doi:10.1016/j.psyneuen.2016.04.001

32. Xie P, Kranzler HR, Poling J, Stein MB, Anton RF. Interaction of FKBP5 with Childhood Adversity on Risk for Post-Traumatic Stress Disorder. *Neuropsychopharmacology*. 2010;35:1684-1692. doi:10.1038/npp.2010.37

33. Ramo-fernández L, Boeck C, Koenig AM, et al. The effects of childhood maltreatment on epigenetic regulation of stress-response associated genes : an intergenerational approach. *Sci Rep*. 2019;9(983):1-12. doi:10.1038/s41598-018-36689-2

34. Kang J, Kim T, Choi J, So H, Kim S. Allele-specific DNA methylation level of FKBP5 is associated with post- traumatic stress disorder. *Psychoneuroendocrinology*. 2019;103(November 2018):1-7. doi:10.1016/j.psyneuen.2018.12.226

35. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene – childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41. doi:10.1038/nn.3275

36. Yehuda R, Mcfarlane A, Vermetten E, Lanius R. Post-traumatic stress disorder. *Nat Rev*. 2015;1(October):1-22. doi:10.1038/nrdp.2015.57

37. Matosin N, Halldorsdottir T, Binder EB. Understanding the Molecular Mechanisms Underpinning Gene by Environment Interactions in Psychiatric Disorders: The FKBP5 Model. *Biol Psychiatry*. 2018;83(10):821-830. doi:10.1016/j.biopsych.2018.01.021

38. Ullman S, Filipas H, Townsend S, Starzynski L. Psychosocial correlates of PTSD symptom severity in sexual assault survivors. *J Trauma Stress*. 2007;20(5):821-831. doi:10.1002/jts.20290

39. Bryant-Davis T, Ullman SE, Tsong Y, Tillman S, Smith K. Struggling to survive: Sexual assault, poverty, and mental health outcomes of african american women. *Am J Orthopsychiatry*. 2010;80(1):61-70. doi:10.1111/j.1939-0025.2010.01007.x

40. Nishith P, Mechanic MB, Resick PA. Prior interpersonal trauma: The contribution to current PTSD symptoms in female rape victims. *J Abnorm Psychol*. 2000;109(1):20-25. doi:10.1037/0021-843X.109.1.20

41. Ullman SE, Najdowski CJ, Filipas HH. Child sexual abuse, post-traumatic stress disorder, and substance use: Predictors of revictimization in adult sexual assault

survivors. *J Child Sex Abus*. 2009;18(4):367-385. doi:10.1080/10538710903035263

42. Campbell R, Greeson MR, Bybee D, Raja S. The Co-Occurrence of Childhood Sexual Abuse, Adult Sexual Assault, Intimate Partner Violence, and Sexual Harassment: A Mediational Model of Posttraumatic Stress Disorder and Physical Health Outcomes. *J Consult Clin Psychol*. 2008;76(2):194-207. doi:10.1037/0022-006X.76.2.194

43. Smoller JW. The Genetics of Stress-Related Disorders: PTSD, Depression, and Anxiety Disorders. *Neuropsychopharmacology*. 2016;41(1):297-319. doi:10.1038/npp.2015.266

44. Wang Q, Shelton RC, Dwivedi Y. Interaction between early-life stress and FKBP5 gene variants in major depressive disorder and post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2018;225(July 2017):422-428. doi:10.1016/j.jad.2017.08.066

45. Paakinaho V, Makkonen H, Jääskeläinen T, Palvimo JJ. Glucocorticoid receptor activates poised FKBP51 locus through long-distance interactions. *Mol Endocrinol*. 2010;24(3):511-525. doi:10.1210/me.2009-0443

46. Yehuda R, Daskalakis NP, Bierer LM, et al. Archival Report Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. *Biol Psychiatry*. 2016;80(5):372-380. doi:10.1016/j.biopsych.2015.08.005

47. Bishop JR, Lee AM, Mills LJ, et al. Methylation of FKBP5 and SLC6A4 in Relation to Treatment Response to Mindfulness Based Stress Reduction for Posttraumatic Stress Disorder. *Front Psychiatry*. 2018;9(September):1-11. doi:10.3389/fpsyt.2018.00418

48. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296

49. Sheehan D, Yves L, Sheehan K, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59(20):34-57.

50. Lecrubier Y, Sheehan D, Weiler E, et al. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity

according to the CIDI. *Eur Psychiatry*. 1997;12(5):224-231.

51. Marques J, Zuardi A. Validity and applicability of the Mini International Neuropsychiatric Interview administered by family medicine residents in primary health care in Brazil. *Gen Hosp Psychiatry*. 2008;30(4):303-310.

52. Davidson JRT, Book SW, Colket JT, et al. Assessment of a new self-rating scale for post-traumatic stress disorder. *Psychol Med*. 1997;27(1):153-160. doi:10.1017/S0033291796004229

53. McDonald S, Beckham J, Morey R, Calhoun P. The validity and diagnostic efficiency of the Davidson Trauma Scale in military veterans who have served since September 11th 2001. *J Anxiety Disord*. 2009;23(2):247-255. doi:10.1038/jid.2014.371

54. Chirwa ED, Sikweyiya Y, Addo-Lartey AA, et al. Prevalence and risk factors of physical or sexual intimate violence perpetration amongst men in four districts in the central region of Ghana: Baseline findings from a cluster randomised controlled trial. *PLoS One*. 2018;13(3):1-18. doi:10.1371/journal.pone.0191663

55. Bernstein D, Fink L. *Childhood Trauma Questionnaire: A Retrospective Self-Report Manual*. San Antonio: The Psychological Corporation; 1998.

56. Jewkes R, Nduna M, Levin J, et al. A cluster randomized-controlled trial to determine the effectiveness of Stepping Stones in preventing HIV infections and promoting safer sexual behaviour amongst youth in the rural Eastern Cape, South Africa: Trial design, methods and baseline findings. *Trop Med Int Heal*. 2006;11(1):3-16. doi:10.1111/j.1365-3156.2005.01530.x

57. Weathers F, Blake D, Schnurr P, Kaloupek D, Marx B, Keane T. *The Life Events Checklist for DSM-5 (LEC-5)*. USA: National Center for PTSD; 2013.

58. Saunders J, Aasland O, Babor T, De La Fuente J, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction*. 1993;88(6):791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

59. Bush K, Kivlahan D, McDonnel M, Fihn S, Bradley K. The AUDIT Alcohol Consumption Questions (AUDIT-C). *Arch Intern Med*. 1998;158(8):1789-1795. doi:10.1097/00000374-199811000-00034

60. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. *J Stud Alcohol*. 1995;56(4):423-432. doi:10.15288/jsa.1995.56.423

61. Cherpitel CJ, Clark WB. Ethnic Differences in Performance of Screening Instruments for Identifying Harmful Drinking and Alcohol Dependence in the Emergency Room. *Alcohol Clin Exp Res*. 1995;19(3):628-634. doi:10.1111/j.1530-0277.1995.tb01559.x

62. Chinyadza E, Moyo IM, Katsumbe TM, et al. Alcohol problems among patients attending five primary health care clinics in Harare city. *Cent Afr J Med*. 1993;39(2):26-32. http://www.ncbi.nlm.nih.gov/pubmed/8261500.

63. Leung SF, Arthur D. The alcohol use disorders identification test (AUDIT): validation of an instrument for enhancing nursing practice in Hong Kong. *Int J Nurs Stud*. 2000;37(1):57-64. doi:10.1016/S0020-7489(99)00052-8

64. Medina-Mora E, Carreño S, De la Fuente JR. Experience with the Alcohol Use Disorders Identification Test (AUDIT) in Mexico. In: *Recent Developments in Alcoholism*. Boston, MA: Springer US; 1998:383-396. doi:10.1007/0-306-47148-5_19

65. Seale JP. Prevalence of Problem Drinking in a Venezuelan Native American Population. *Alcohol Alcohol*. 2002;37(2):198-204. doi:10.1093/alcalc/37.2.198

66. Simbayi L, Kalichman S, Jooste S, Mathiti V, Cain D, Cherry C. Alcohol use and sexual risks for HIV infection among men and women receiving sexually transmitted infection clinic services in Cape Town, South Africa. *J Stud Alcohol*. 2004;65:434-442.

67. Radloff L. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas*. 1977;1(3):385-401. doi:10.1002/ardp.19552881111

68. Boyd JH. Screening for Depression in a Community Sample. *Arch Gen Psychiatry*. 1982;39(10):1195. doi:10.1001/archpsyc.1982.04290100059010

69. Eller LS, Mahat G. Psychological factors in Nepali former commercial sex workers with HIV. *J Nurs Scholarsh*. 2003;35(1):53-60. doi:10.1111/j.1547-5069.2003.00053.x

70. Ghubash R, Daradkeh TK, Al Naseri KS, Al Bloushi NBA, Al Daheri AM. The

performance of the center for epidemiologic study depression scale (CES-D) in an Arab female community. *Int J Soc Psychiatry*. 2000;46(4):241-249. doi:10.1177/002076400004600402

71. Guarnaccia PJ, Angel R, Worobey JL. The factor structure of the CES-D in the Hispanic health and nutrition examination survey: The influences of ethnicity, gender and language. *Soc Sci Med*. 1989;29(1):85-94. doi:10.1016/0277-9536(89)90131-7

72. Iwata N, Buka S. Race/ethnicity and depressive symptoms: a cross-cultural/ethnic comparison among university students in East Asia, North and South America. *Soc Sci Med*. 2002;55(12):2243-2252. doi:10.1016/S0277-9536(02)00003-5

73. Munet-Vilaró F, Folkman S, Gregorich S. Depressive Symptomatology in Three Latino Groups. *West J Nurs Res*. 1999;21(2):209-224. doi:10.1177/01939459922043848

74. Myers J, Weissman M. Use of a self-report symptom scale to detect depression in a community sample. *Am J Psychiatry*. 1980;137(9):1081-1084. doi:10.1176/ajp.137.9.1081

75. Roberts RE, Rhoades HM, Vernon SW. Using the CES-D scale to screen for depression and anxiety: Effects of language and ethnic status. *Psychiatry Res*. 1990;31(1):69-83. doi:10.1016/0165-1781(90)90110-Q

76. Roberts R, Vernon S. The Center for Epidemiologic Studies Depression Scale: its use in a community sample. *Am J Psychiatry*. 1983;140(1):41-46. doi:10.1176/ajp.140.1.41

77. Simoni JM, Ng MT. Trauma, coping, and depression among women with HIV/AIDS in New York City. *AIDS Care - Psychol Socio-Medical Asp AIDS/HIV*. 2000;12(5):567-580. doi:10.1080/095401200750003752

78. Claus R, Wilop S, Hielscher T, et al. A systematic comparison of quantitative high-resolution DNA methylation analysis and methylation-specific PCR. *Epigenetics*. 2012;7(7):772-780. doi:10.4161/epi.20299

79. Ehrich M, Nelson MR, Stanssens P, et al. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry. *Proc Natl Acad Sci U S A*. 2005;102(44):15785-15790. doi:10.1073/pnas.0507816102

80. Binder EB, Salyakina D, Lichtner P, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet*. 2004;36(12):1319-1325. doi:10.1038/ng1479

81. de Kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, Westenberg HGM. Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. *J Psychiatr Res*. 2006;40(6):550-567. doi:10.1016/j.jpsychires.2005.08.002

82. Binder EB. Association of <emph type="ital">FKBP5</emph> Polymorphisms and Childhood Abuse With Risk of Posttraumatic Stress Disorder Symptoms in Adults. *JAMA*. 2008;299(11):1291. doi:10.1001/jama.299.11.1291

83. Wang H, Lou D, Wang Z. Crosstalk of genetic variants, allele-specific DNA methylation, and environmental factors for complex disease risk. *Front Genet*. 2019;10(JAN):1-15. doi:10.3389/fgene.2018.00695

84. Romanowska J, Haaland ØA, Jugessur A, et al. Gene-methylation interactions: Discovering region-wise DNA methylation levels that modify SNP-associated disease risk. *Clin Epigenetics*. 2020;12(1):1-18. doi:10.1186/s13148-020-00881-x

85. Galanter JM, Gignoux CR, Oh SS, et al. Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. *Elife*. 2017;6:e20532. doi:10.7554/eLife.20532

86. Xia Y yin, Ding Y bing, Liu X qing, et al. Racial/ethnic disparities in human DNA methylation. *Biochim Biophys Acta*. 2014;1846:258-262. doi:10.1016/j.bbcan.2014.07.001

87. Chung WCJ, Auger AP. Gender differences in neurodevelopment and epigenetics. *Eur J Physiol*. 2013;465(5):573-584. doi:10.1007/s00424-013-1258-4

88. Speer KE, Semple S, Naumovski N, D'Cunha NM, McKune AJ. HPA axis function and diurnal cortisol in post-traumatic stress disorder: A systematic review. *Neurobiol Stress*. 2019;11(December 2018):100180. doi:10.1016/j.ynstr.2019.100180

89. Tyrka A, Burgers D, Philip N, Price L, Carpenter L. The neurobiological correlates of childhood adversity and implications for treatment. *Acta Psychiatr Scand*. 2013;128:434-447. doi:10.1111/acps.12143

90. Segerstrom SC. Statistical Guideline #1. Avoid Creating Categorical Variables from Continuous Variables. *Int J Behav Med*. 2019;26(4):329-330. doi:10.1007/s12529-019-09790-7

91. Riggs SA. Childhood Emotional Abuse and the Attachment System Across the Life Cycle: What Theory and Research Tell Us. *J Aggress Maltreat Trauma*. 2010;19(1):5-51. doi:10.1080/10926770903475968

92. Pechtel P, Pizzagalli D. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology (Berl)*. 2011;214(1):55-70.

93. Post LM, Zoellner LA, Youngstrom E, Feeny NC. Understanding the relationship between co-occurring PTSD and MDD: Symptom severity and affect. *J Anxiety Disord*. 2011;25(8):1123-1130. doi:10.1016/j.janxdis.2011.08.003

94. Taft CT, Resick PA, Watkins LE, Panuzio J. An investigation of posttraumatic stress disorder and depressive symptomatology among female victimsof interpersonal trauma. *J Fam Violence*. 2009;24(6):407-415. doi:10.1007/s10896-009-9243-6

95. Nixon RD V, Resick PA, Nishith P. An exploration of comorbid depression among female victims of intimate partner violence with posttraumatic stress disorder. *J Affect Disord*. 2004;82(2):315-320. doi:10.1016/j.jad.2004.01.008

96. Wilson SM, Burroughs TK, Newins AR, et al. The association between alcohol consumption, lifetime alcohol use disorder, and psychiatric distress among male and female veterans. *J Stud Alcohol Drugs*. 2018;79(4):591-600. doi:10.15288/JSAD.2018.79.591

97. McFarlane AC, Browne D, Bryant RA, et al. A longitudinal analysis of alcohol consumption and the risk of posttraumatic symptoms. *J Affect Disord*. 2009;118(1-3):166-172. doi:10.1016/j.jad.2009.01.017

98. Tipps ME, Raybuck JD, Lattal KM. Neurobiology of Learning and Memory Substance abuse , memory , and post-traumatic stress disorder. *Neurobiol Learn Mem*. 2014;112:87-100. doi:10.1016/j.nlm.2013.12.002

99. Lee RS, Tamashiro KLK, Yang X, et al. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice.

Endocrinology. 2010;151(9):4332-4343. doi:10.1210/en.2010-0225

100. Ewald ER, Wand GS, Seifuddin F, et al. Alterations in DNA methylation of Fkbp5 as a determinant of blood-brain correlation of glucocorticoid exposure. *Psychoneuroendocrinology*. 2014;44:112-122. doi:10.1016/j.psyneuen.2014.03.003

101. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13(1). doi:10.1186/1471-2105-13-86

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. SUMMARY

The main aim of this study was to investigate the pre-, peri- and post-trauma risk and protective factors related to socio-demographic, psychological and epigenetic mechanisms in the aetiology and trajectory of posttraumatic stress disorder (PTSD) following rape-exposure. Socio-economic factors included demographic factors such as age, gender, relationship status and income/employment status, as well as social factors such as social support and stigma associated with rape. Psychological factors included exposure to trauma, resilience, perceived stress, alcohol use and depression. Epigenetic mechanisms included epigenome-wide differential methylation of genes in relation to PTSD status at 3-months post-rape as well as the longitudinal change in brain-specific serine/threonine-protein kinase *(BRSK2)*, pituitary adenylate cyclase-activating polypeptide 1 *(ADCYAP1)* and FK506 binding protein *(FKBP5)* methylation levels in relation to longitudinal changes in PTSD symptom scores. A common functional single nucleotide polymorphism (SNP) of the *FKBP5* gene was also investigated, given that it has been associated with biological changes in the functioning of the stress response following childhood trauma exposure and increases the risk for psychiatric disorders following adult revictimization [1–3]. A systematic review of epigenetic changes in relation to childhood trauma across adult onset psychiatric disorders was also conducted given that little is known about the shared epigenetic changes resulting from childhood trauma across psychiatric disorders [4].

The parent study, the Rape Impact Cohort Evaluation (RICE) [5] allowed for the successful assessment and collection of peripheral blood DNA samples from rape-exposed women. A relatively homogenous cohort (similar socio-demographic background, similar history of trauma exposure and aged between 16 and 40 years; n = 852) of rape-exposed women were assessed at baseline (within 20 days of the rape), 3-months and 6-months post-rape. The large cohort investigated in the parent study allowed us to control for various confounding factors associated with DNA methylation including the exclusion of women who were pregnant or lactating, women who met criteria for PTSD at the baseline assessment indicating PTSD due to a past traumatic event other than the rape, and women who had HIV seroconverted. We also matched participants as closely as possible on HIV status, age, education level, income, body mass index (BMI), smoking status and lifetime trauma exposure.

To the best of our knowledge, this is the first study to compare epigenome-wide DNA methylation profiles between rape-exposed women with and without PTSD. It is also the first study to investigate epigenome-wide methylation profiles in women of African ethnicity. There

are no known studies that have investigated longitudinal change in methylation in relation to change in PTSD scores in rape-exposed women exclusively. Longitudinal studies comprehensively investigating pre-, peri- and post-rape psychosocial risk and protective associated with PTSD do exist but are scarce, especially those investigating PTSD beyond 3-months post-rape and studies solely investigating rape as opposed to the broader category of sexual assault [6]. This study therefore makes an important contribution to understanding the psychosocial risk and protective factors associated with mental health in the aftermath of rape. Identifying epigenetic differences and changes over time in relation to PTSD further improves our understanding of the molecular mechanisms mediating the association between environmental exposures and mental health outcomes.

A comprehensive discussion of the findings related to each objective of this study is presented in the chapters dedicated to each objective (chapter 2-5). An overview of the key findings, contribution of the findings to the knowledge gaps, limitations of the study, concluding remarks, recommendations for practice and recommendations for future research are discussed below.

6.2. OVERVIEW OF KEY FINDINGS

6.2.1. Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review

Childhood trauma is prevalent across low-, middle- and high-income countries and increases the risk for nearly all adult-onset psychiatric disorders as well as suicide [7–11]. Differential methylation of genes associated with the biological response to stress and trauma may underlie a range of phenotypic presentation, including behavioural and affective symptoms [12,13]. The epigenome is particularly amenable to environmental influences during childhood, given heightened brain plasticity during critical developmental periods [14,15]. Studies have investigated the relationship between differential methylation and childhood trauma in relation to adult-onset psychiatric disorders, but little is known about the discrepancies and commonalities in pathways affected by differential methylation across psychiatric disorders [14,16]. We conducted a systematic review investigating differential methylation in relation to childhood trauma and adult mental health outcomes and determined the following:

(1) high levels of childhood trauma were associated with increased *NR3C1* methylation in mood and personality disorders and decreased methylation in anxiety and trauma-related disorders;

(2) high levels of childhood trauma were associated with increased *BDNF* methylation in healthy adult and adults with borderline personality disorder;
(3) high levels of childhood trauma were associated with increased *OXTR* methylation in healthy adults;
(4) studies investigating the relationship between differential methylation and childhood trauma were generally limited by a lack of statistical power, stemming from small sample sizes;
(5) there was a lack of uniformity in defining childhood trauma and some studies did not use standardised measures to assess childhood trauma exposure;
(6) there was a lack of uniformity in the molecular methods used to investigated differential methylation;
(7) the vast majority of studies followed a candidate gene design and implicated differential methylation at single CpG sites in the relationship between childhood trauma and psychiatric disorders;
(8) the vast majority of studies were conducted in American, Canadian and European countries on Caucasian individual, only one study was conducted in an Asian country and none were conducted in African countries.

The findings provide evidence for the role of *NR3C1*, *BDNF* and *OXTR* in the association between childhood trauma and adult-onset psychiatric disorders. *NR3C1*, *BDNF* and *OXTR* have all been implicated in the functioning of the hypothalamic-pituitary-adrenal (HPA) axis and the neuroendocrinology of psychiatric disorders [6,17]. The findings highlight the need to refine the design and methodological strategies used in epigenetic studies to eliminate the noise caused by confounding factors [18]. Identifying discrepancies and commonalities in pathways affected by childhood trauma across psychiatric disorders may aid in identifying biomarkers of psychiatric disorders and developing epigenetic therapies [18–20].

6.2.2. Risk and protective factors affecting the symptom trajectory of posttraumatic stress disorder post-rape

The prevalence of PTSD in sexual assault survivors (35% - 45% at 3-months post-rape) is higher than those associated with other trauma types [17,21–23]. Risk and protective factors for PTSD in sexual assault survivors have been found to differ from those associated with non-sexual trauma types [6,22]. Putative risk and protective factors for PTSD following rape include pre-assault factors (e.g. age [22,24,25], relationship status [17,25,26], education [24,26]

employment/income [17,24,25], childhood trauma exposure [26–28] adult cumulative lifetime trauma [17,22,26] and HIV status[29]), assault factors (e.g. multiple perpetrators [17], multiple sexual acts [17], physical injury inflicted during the rape [17,25] and a longer duration of assault [30]) and post-assault factors (e.g. resilience[22,26,31], social support[32], perceived stress [33,34], rape stigma[25,26,31,35], alcohol use, depression[17,24,27,36–41]). The aforementioned risk and protective factors may also have long-term effects on the trajectory of PTSD symptomatology [6,34,42]. Few studies have comprehensively investigated pre- and post-assault risk and protective factors for PTSD in a longitudinal prospective design, especially beyond the 3-month post-rape period and in low- to medium-income countries [6,17]. We investigated pre-assault, assault and post-assault risk factors as predictors of the trajectory of PTSD symptoms over 6-months (n = 639) and determined the following:

(1) baseline age, education, relationship status, employment status, HIV status, childhood trauma and lifetime trauma exposure were not significant predictors of PTSD at any timepoint;
(2) if the perpetrator was known to the rape survivor or was a stranger, being abducted or lured somewhere under false pretences or not, the use of a weapon or not, use of bodily physical force or not, if the perpetrator or accomplice threatened to kill the rape survivor or not, if the rape survivor thought she would be killed or not, if the same perpetrator/s had raped the same survivor on a previous occasion or not, the number of different sexual acts that occurred during the rape (vaginal/anal/oral/digital/object penetration, forced or observed masturbation); and if the rape was reported to the police or not were not significant predictors of PTSD at any timepoint;
(3) rape survivors reporting a single perpetrator had higher PTSD scores at 3-months post-rape compared to those who reported multiple perpetrators;
(4) baseline resilience, social support, perceived stress and alcohol use were not significant predictors of PTSD scores over time;
(5) increased baseline depression scores predicted increased PTSD scores at 3-months and 6-months post-rape;
(6) increased baseline rape stigma scores predicted increased PTSD scores at 6-months post-rape.

The findings support those reported in previous studies where increased baseline depression [17,27,43] and rape stigma had a significant adverse effect on PTSD recovery following rape. [25,26,31,35]. The finding that rape involving a single perpetrator (compared to multiple

perpetrators) is associated with increased PTSD scores at 3-months post-rape is counterintuitive and needs further investigation. Screening for depression and identifying and correcting stigmatising views associated with rape soon after the rape occurred may have long-term beneficial effects.

6.2.3. Genome-wide differentially methylated genes associated with posttraumatic stress disorder and longitudinal change in methylation in female rape survivors

Epigenetic mechanisms, including DNA methylation, are known to respond to environmental exposures such as trauma and can lead to stable changes in gene expression [44,45]. DNA methylation responses may confer risk or protection for PTSD, as they may alter an individual's ability to adapt to traumatic events on a molecular level [46,47] To date, twelve epigenome-wide association studies (EWASs) investigating blood DNA methylation differences between PTSD cases and controls have been published [48–56], but none of the gene-specific findings have been replicated between EWASs. The lack of replication may be due to design limitations and demographic differences across studies. We conducted an EWAS (n = 48) in a demographically similar group of rape-exposed women and attempted to validate and replicate findings related to selected genes associated with PTSD in the EWAS. We determined the following:

(1) One differentially methylated position (DMP, cg01700569) was associated with PTSD after correction for multiple testing; this CpG site was located in an intergenic region and has not been associated with any phenotypes or behaviours in previous studies;
(2) Thirty-four differentially methylation regions (DMRs) were associated with PTSD and included CpG regions in *BRSK2* and *ADCYAP1*; *BRSK1*, a paralog of *BRSK2*, and *ADCYAP1* receptor 1 *(ADCYAP1R1)* have been linked to the development of PTSD in prior studies [51,57–59]
(3) The *BRSK2* region (chr11:1463541-1463670) identified from the EWAS included five CpG sites (CpG1 - cg12186219, CpG2 - cg14064268, CpG3 - cg10590925, CpG4 - cg17429870, CpG5 - cg18651858) that showed decreased methylation in participants with PTSD;
(4) The *ADCYAP1* region (chr18:905177-905180) identified from the EWAS included two CpG sites (CpG1 – cg22388954, CpG2 – cg11773720) that showed increased methylation in participants with PTSD.
(5) In the validation analysis related to *BRSK2*, methylation levels of CpG3 and CpG4 at 3-months post-rape were not significantly associated with PTSD status at 3-months

post-rape. Decreased methylation of CpG5 at 3-months post-rape was significantly associated with a PTSD status at 3-months post-rape, but the association was no longer significant when covariates were added to the model.

(6) In the validation analysis related to *ADCYAP1*, methylation levels of CpG1&2 was not significantly associated with PTSD status at 3-months post-rape.

(7) In the replication analysis related to *BRSK2*, methylation levels of *BRSK2* CpG3, CpG4 and CpG5 were not significantly associated with PTSD status at 3-months post-rape.

(8) In the replication analysis related to *ADCYAP1*, methylation levels of *ADCYAP1* CpG1&2 were not significantly associated with PTSD status at 3-months post-rape.

The EWAS was followed by a longitudinal investigation of *BRSK2* and *ADCYAP1* methylation levels as predictors of PTSD symptom scores at baseline, 3-months and 6-months post-rape. We determined the following:

(9) decreased baseline *BRSK2* CpG3, CpG4 and CpG5 methylation levels were associated with increased PTSD scores at 3- and 6-months post-rape. Decreased *BRSK2* methylation at 3-months and 6-months post-rape was associated with increased PTSD scores at the same time-points. However, the relationship between decreased *BRSK2* CpG3 methylation at 3-months post-rape and increased PTSD scores at 3-months post-rape was the only association that remained significant after covariates were added to the models.

(10) decreased baseline *ADCYAP1* CpG1&2 methylation was associated with increased PTSD scores at 6-months post-rape. Decreased *ADCYAP1* methylation at 3-months and 6-months post-rape was associated with increased PTSD scores at the same time-points, while decreased baseline *ADCYAP1* CpG1&2 methylation was associated with decreased PTSD scores at baseline. The findings did not remain significant after PTSD covariates were added to the models.

The findings provide evidence for the role of *BRSK2* and *ADCYAP1* in the development and maintenance of PTSD symptoms over time. Differential methylation of *BRSK2* may contribute to adverse neuronal development, neuronal maintenance and dysregulated blood glucose levels, which may explain the increased risk for diabetes and cardiovascular disease observed in prior PTSD studies [60,61]. Differential methylation of *ADCYAP1* may result in a dysregulated

HPA-axis since its protein product PACAP is a master regulator of the HPA-axis [62,63]. A dysregulated HPA-axis is a well-known phenomenon in PTSD [62–64].

6.2.4. *FKBP5* intron 7 methylation and the trajectory of PTSD symptoms in rape-exposed women

Most studies investigating differential methylation in relation to PTSD have focused on genes that are directly or indirectly associated with regulation of the HPA-axis [16,47]. One of the most commonly investigated genes is the FK506 binding protein gene *(FKBP5)* and a SNP within the gene, rs1360780 [47,65]. The interaction between childhood trauma and the rs1360780 T allele has previously been associated with an increased risk for the development of PTSD [1,16,47,66,67] and with differential *FKBP5* intron 7 methylation [3,68–71]. Only one study has investigated longitudinal change in intron 7 methylation in relation to change in PTSD symptom scores in a war veteran sample, without considering the contribution of rs1360780 genotype [70]. We investigated the association between longitudinal *FKBP5* methylation and PTSD symptoms (baseline, 3-months and 6-months post-rape) while also considering childhood trauma and rs1360780 genotype. We determined the following:

(1) Decreased methylation of *FKBP5* CpG sites was associated with increased PTSD scores at 3-months and 6-months post-rape.
(2) Decreased methylation of one *FKBP5* CpG site remained a significant predictor of increased PTSD scores at 3-months post-rape after adjusting for depression and alcohol use.
(3) Higher childhood trauma was a predictor of increased PTSD scores at baseline.
(4) Increased baseline *FKBP5* methylation at two CpG sites was associated with increased baseline PTSD scores in the group with high childhood trauma and carrying the CC genotype of rs1360780, but the interaction effect was no longer significant when baseline depression and alcohol use were included as covariates in the model.

The findings provide evidence for the role of *FKBP5* intron 7 methylation in the development and course of PTSD over six months. The findings also confirm the strong relationship between childhood trauma and PTSD [26–28] as well as the strong relationship between depression and PTSD reported in prior studies [72,73]. Decreased methylation is likely to result in overexpression of *FKBP5* which may disrupt the ultra-short intracellular negative feedback loop regulated by the interaction between glucocorticoid receptor and *FKBP5* [74,75]. Differential methylation of

FKBP5 and other HPA-axis associated genes may mediated the relationship between HPA-axis dysfunction and increased risk for PTSD [76,77].

6.2. CONTRIBUTION TO THE KNOWLEDGE GAPS

First, the study provides synthesised evidence for the role of *NR3C1* in mediating the association between childhood trauma and increased risk for the development of adult onset psychiatric disorders. We also identified that the direction of *NR3C1* methylation (increased/ decreased) is dependent on psychiatric diagnosis [78–85].

Second, we identified several discrepancies in methodological strategies applied when investigating differential methylation in relation to childhood trauma and adult onset psychiatric disorders. These discrepancies may hinder the pursuit to finding commonalities in pathways affected by childhood trauma across psychiatric disorders [18–20].

Third, although prior studies have reported a link between depression, rape stigma and PTSD in sexual assault survivors, few have investigated their effect on long-term recovery following rape [17,23]. The majority of longitudinal rape studies have been conducted in high-income countries that are better equipped to provide psychological and medical support to rape survivors suffering from PTSD compared to low-resourced countries [86,87]. Identifying risk factors for the development and trajectory of PTSD in low- and middle-income countries is important since they may differ from those reported in high-income countries and may aid in developing context-specific evidence-based health care [88]. Early intervention in countries characterised by limited resources and overburdened mental health care facilities is also important since treatment of severe psychopathology is more resource intensive (e.g. longer duration of treatment, medical interventions, hospitalisation, management of comorbid disorders) with higher costs and burden for the healthcare system [86,87].

Forth, the current study is the first to investigate genome-wide differentially methylated genes associated with PTSD in an African country. The majority of prior EWASs have investigated differential methylation in North American participants [89,90]. Studying differential methylation in and between different ethnicity groups is important given that shared environmental exposures and ethnicity-specific evolution may influence methylation profiles [91,92]. Differences in methylation profiles between ethnicity groups may also increase the risk for comorbid psychiatric disorders and physiological diseases [92,93].

Fifth, the current study is also the first to investigate genome-wide differentially methylated genes associated with PTSD in a rape-exposed sample. The majority of prior EWASs have investigated combat-exposed populations and civilian samples exposed to a mixture of trauma types [47,89,90]. Rape is associated with a high risk for the development of PTSD compared to other trauma types and methylation profiles of rape-exposed women may differ from those associated with other trauma types [94,95].

Sixth, other than ethnicity and trauma exposure, we also controlled for the effect of various other variables that may influence methylation profiles. We investigated differential methylation associated with rape in women only, since gender may influence methylation profiles and differential methylation may explain the increased risk for PTSD observed among women [96]. Epigenetic differences governing sexual differentiation (e.g. testosterone, estrogen, steroid receptors, binding proteins) and their impact on neurological and endocrine functioning may mediate the risk for developing PTSD [97,98].

We investigated methylation profiles in women of similar age (16 – 40 years), thereby controlling for differential methylation related to chronological age which may underly the phenotypic presentation of aging and age associated increased risk for diseases [99–104].

Differential methylation governs aspects of pregnancy and lactation [105]. To avoid confounding, we excluded women who were pregnant or lactating from the methylation analyses.

We investigated methylation differences in blood samples, which reflect methylation in brain regions associated with PTSD more accurately than saliva and buccal mucosa samples [106]. All prior EWASs investigating PTSD have used blood samples, which allows collating findings and identify potential blood biomarkers (since brain tissue cannot be used to assess risk) of psychiatric disorders and using this information to prioritise and individualise treatment according to individual risk profiles [107] Blood cell type composition was estimated by applying the Houseman algorithm to DNA methylation profiles using a publicly available blood cell type reference dataset (Gene Expression Omnibus accession number GSE35069) [108]. We therefore controlled for differential methylation associated with the cell type composition in whole blood [109,110].

Pharmacotherapy for PTSD, including psychotropic medication, have been found to exert their effects through alterations in DNA methylation, chromatin structure, transcription factor activity and receptor expression [111]. None of the participants included in the methylation

analyses were in receipt of psychotropics. A number of participants used antiretrovirals (ARV) and medication for sexually transmitted infections (STIs) and a few were on medication for hypertension. We included medication use in the methylation analyses to control for the effect of medication use on methylation profiles. We did not include contraception as a covariate since the majority of women were using hormone contraceptives.

Cigarette smoke and alcohol dependence have been associated with reduced global methylation and genome-wide differential methylation profiles [112–114]. The confounding effects of smoking and alcohol use was controlled for by included these factors in the methylation analyses.

Differential methylation of genes associated with transcription factors, chromatin remodelling, viral binding and viral transport have been associated with HIV infection as well as genes associated with the immune, metabolic and endocrine system [115–118]. There was a high HIV prevalence rate in our sample, and we included HIV status as a confounding variable in the methylation analysis.

PTSD is associated with an increased risk for metabolic disease including obesity, type 2 diabetes, hypertension, increased triglycerides and decreased high-density lipoprotein (HDL) cholesterol [119,120]. The link between PTSD and metabolic disease is likely due to differential methylation of genes involved in mitochondrial function, metabolic and endocrine processes shared by both conditions [121]. We included BMI, as a proxy measure for metabolic syndrome, as a confounding factor in the methylation analyses.

Childhood trauma is associated with an increased risk for PTSD and with genome-wide differential methylation [122–124]. Childhood trauma was included as a covariate when investigating psychosocial risk and protective factors for PTSD and in the methylation analyses.

Seventh, the study provides evidence for the role of differential methylation of *BRSK2* in the development and maintenance of PTSD symptoms over time. Differential methylation of *BRSK2* may also contribute to the PTSD-associated increased risk for metabolic syndrome given that *BRSK2* has been linked to metabolic processes and glucose homeostasis [125,126] Interestingly, *BRSK1* a paralog of *BRSK2*, was found to be differentially methylated in a prior EWAS investigating male Vietnam war veterans [51].

Eight, the study also provides evidence for the role of differential methylation of *ADCYAP1* in the development and maintenance of PTSD symptoms over time. This finding builds on prior studies investigating HPA-axis dysregulation in relation to PTSD, given that *ADCYAP1*'s protein product, PACAP, is an important regulator of the HPA-axis and the stress response [62]. Our findings are also supported by previous research implicating *ADCYAP1/ADCYAP1R1* methylation and expression (especially in women) in the development of PTSD [57–59,70,127].

Ninth, the current study is the second study to investigate longitudinal change in *FKBP5* intron 7 methylation in relation to change in PTSD symptom scores [70] and the first to investigated *FKBP5* intron 7 methylation in a rape-exposed African sample. Our finding supports those reported in the prior longitudinal *FKBP5* intron 7 methylation studies since decreased methylation of *FKBP5* CpG sites were associated with increased PTSD scores in our study and the prior study [70]. Our findings further support prior findings investigating HPA-axis dysregulation in relation to PTSD given that *FKBP5* is a coregulator of the glucocorticoid receptor which signals the activation of the negative feedback loop of the HPA-axis [1–3,128,129].

6.3. LIMITATIONS

Some limitations were unavoidable in this study and deserve mention. First, all participants included in this study disclosed the rape and sought help from rape crisis centres. Underreporting of rape is common and may in itself be influenced by sociodemographic and psychosocial risk and protective factors. As such, we could not assess risk and protective factors associated with PTSD in non-disclosing rape survivors.

Second, some participants received counselling at the rape crisis centres at which they presented and/or at the study site. We could not include counselling as a predictor of PTSD scores in the multivariate analyses since we did not have this information for those who were lost to follow-up.

Third, although PTSD and depression are considered distinct disorders, some of the symptoms of depression do overlap with the symptoms of PTSD [130]. Depression may explain a significant proportion of variance in PTSD simply based on overlapping symptom constructs, in which case it cannot be considered a predictor of PTSD, but rather a symptom domain of PTSD [131–133].

Forth, baseline depression and alcohol use were investigated as post-assault risk factors for PTSD, assuming that they develop and covary with PTSD symptoms. Although depression and alcohol use often covary with PTSD symptoms following rape, they may also be pre-existing factors that increase the risk for sexual assault and PTSD post-rape [17,24,135,27,36–41,134].

Fifth, the Mini International Neuropsychiatric Interview (MINI) was used to exclude participants who met criteria for a PTSD diagnosis at baseline, due to an event other than the rape. The MINI is a clinician administered diagnostic tool and should ideally be administered by a clinical psychologist or a psychiatrist. The baseline MINI interviews were administered by a registered trauma counsellor in this study and a lack of diagnostic and clinical skills may have resulting in inaccurate diagnoses. Interrater reliability and repeat calibration between the trauma counsellor and the psychiatrist who provided training in administering the MINI was also not assess and would have been beneficial in establishing the reliability and validity of the diagnoses.

Sixth, we could not compare post-rape epigenetic changes to pre-rape epigenetic markers, since we did not have pre-rape data i.e. the baseline assessment was completed within 20 days following the rape. This is a limitation for determining whether a cause-effect relationship exists between rape exposure and epigenetic changes.

Seventh, some of epigenetic changes observed may have occurred due to a history of childhood trauma. Although we controlled for the effect of childhood trauma in the analyses, it is difficult to disentangle the epigenetic changes that occurred as a result of childhood trauma from those that occurred as a direct result of the rape.

Eight, the methylation analyses were likely underpowered given the small sample size. Although increasing the sample size in epigenetic studies does not necessarily increased the power given that the epigenome is not static like the genome [136]. Heterogeneity of the sample may be of equal importance to sample size in epigenetic studies [136].

Ninth, we used whole blood to measure methylation levels while differential methylation in brain tissue are more likely to underly PTSD pathophysiology [107]. However, studies have found that blood-brain methylation levels are often correlated [137,138] and identifying potential blood biomarkers of psychiatric disorders may contribute to prioritising and individualising treatment according to individual risk profiles [107]. Brain tissue cannot be used to identify biomarkers of PTSD since it is not accessible in living participants [107].

Tenth, we used the Houseman algorithm[108] and the publicly available blood cell type reference dataset GSE35069[139] to estimate, and control for, blood cell-type composition at 3-months post-rape, in the EWAS. This was implemented within the *meffil* pipeline. We did not use flow cytometry to identify and count specific blood cell types in individual samples empirically, and we were therefore unable to control for within-individual change in blood cell type composition over time. We also did not have epigenome-wide information for the longitudinal analyses and could not implement the Houseman method to determine cell-type composition. Potential confounding in methylation findings due to change in blood cell-type compositions can therefore not be determined. However, surrogate variable analysis (SVA) was also applied and controls for unwanted variance in methylation caused by biological differences in the sample which are unrelated to the phenotype of interest i.e. PTSD. The surrogate variables were included as covariates in the EWAS models.

Eleventh, DNA methylation was not assessed in relation to RNA expression in this study and conclusions related to the functional effects of methylation are only assumed.

Twelfth, self-report measures were used to assess PTSD symptoms and other psychosocial risk and protective factors. Subjective rating of symptoms may result in over- or under-reporting compared to clinician administered measure which are considered more objective [140].

Thirteenth, multiple comparisons may have increased the risk of Type I errors in some of the analyses and those findings should be considered preliminary findings.

Fourteenth, the self-report measure of perceived stress showed poor reliability in the study and the results related to this measure should be interpreted with caution.

6.4. CONCLUSION

This study demonstrated that depression is a significant risk factors for the development and trajectory of PTSD symptoms post-rape. This finding is similar to those reported in longitudinal rape studies conducted in high-income countries where depression was as prevalent as PTSD following rape, if not more prevalent, especially in the immediate aftermath of rape [17,22,23,30]. Some studies suggest that PTSD and depression remain distinct disorders even though there is an overlap in the symptoms reported between the disorders [72,131–133] while others suggest that PTSD symptoms mediate the relationship between depression and the effect of time [23,130]. There seems to be a uniquely amplified relationship between PTSD and depression in the

aftermath of rape in low- to medium-income and high-income countries compared to the relationship between PTSD and depression related to exposure to other trauma types [17,23,24,72].

Similar to prior longitudinal studies, we also found that protective factors, such as resilience and social support, lose their protective effect when accounting for the effect of depression in relation to PTSD, in the context of rape [22,24]. Prior studies suggest that the physical and emotional intrusive nature of rape results in severe traumatisation and that the severity of the trauma leaves little room for other factors to have an impact on the risk of developing PTSD [22,141]. This finding emphasises the need for emotional support from professional counsellors and mental health facilities given that positive internal coping mechanisms are not sufficient in dealing with the adverse effects of rape [142].

Rape stigma is less commonly investigated in rape studies conducted in high-income countries but those who have investigated elements of rape stigma reported a significant relationship between negative social reactions (e.g. victim blaming, self-blaming, shame, embarrassment, downplaying the severity of the rape, treating the victim differently) and PTSD post-rape [25,26,31,35]. It is plausible that the effect of rape stigma is more pronounced in women from low socioeconomic backgrounds, given that low socioeconomic status is associated with less equitable views of gender roles in both perpetrators and rape survivors [143,144]. Less equitable gender roles and perceptions of gender roles are likely to result in women blaming themselves for the rape and community members downplaying the severity of the rape or justifying the rape through perceptions of male sexual entitlement [143,144].

With regard to methylation findings, we confirmed that genes associated with the HPA-axis are implicated in the development and course of PTSD symptoms post-rape [16,47]. Both *ADCYAP1* and *FKBP5* are associated with HPA-axis functioning and have been implicated in the risk for developing PTSD in prior studies [16,47]. The role of HPA-axis genes in the development of PTSD seems to be independent of ethnicity and the type of trauma endured, given that findings have been replicated in various populations [145]. Based on prior findings, the effect of *ADCYAP1* methylation and expression in relation to PTSD risk in more pronounced in women which highlights the need for gender stratification in methylation studies [58,59,146,147].

Our finding that *BRSK2* in associated with PTSD also builds on a prior finding where genome-wide differential methylation of *BRSK1*, a paralog of *BRSK2*, was associated with PTSD in a study investigating Australian male Vietnam veterans [51]. Differential expression of both *BRSK1* and *BRSK2* have been linked to disorganised presynaptic vesicle formation, uncoordinated release and reuptake of neurotransmitters, altered axonal development and

abnormal neuronal polarisation in animal studies [148–153]. *BRSK2* has also been associated with metabolic processes and glucose homeostasis [125,126]. Further investigation of *BRSK2* in future human studies could potentially increase our understanding of the brain morphology associated with PTSD and may also aid in understanding the relationship between PTSD and metabolic syndrome observed in prior studies [60,61].

In summary, the study builds on the existing literature in highlighting the risk factors for the development and course of PTSD in rape-exposed women. Methylation findings also builds on the existing literature, although the genome-wide finding implicating differential methylation of *BRSK2* in the development of PTSD is a novel finding in human studies. The study provides evidence that both psychological and biological factors have an impact on the symptom trajectory of PTSD post-rape.

6.5. RECOMMENDATIONS FOR PRACTICE

Although *ADCYAP1* and *BRSK2* methylation levels were identified as potential biomarkers of PTSD risk and symptom trajectory, further research is needed to determine if the differentially methylated regions identified in this study are consistently linked to the development of PTSD. Replication of our findings and testing for PTSD associated biomarkers may aid in prioritising treatment according to individual risk profiles [107]. Identifying and replicating findings in studies that possess increased power may also aid in developing targeted pharmaceutical interventions [154].

There is a growing body of literature linking differential methylation, expression and SNPs in *FKBP5* to the altered neurocircuitry and brain morphology observed in PTSD and other psychiatric disorders [67]. Human studies have shown that higher expression of *FKBP5* increases the risk for psychiatric disorders and manipulating *Fkbp5* in rodents has resulted in reduced anxiety, increased stress coping, increased glucocorticoid receptor sensitivity and enhanced functioning of the negative feedback loop of the HPA-axis [155,156]. Increased expression of *FKBP5* following exposure of adipose tissue to dexamethasone treatment has also provided evidence for the for the role of *FKBP5* in stress induced metabolic dysregulation [157]. FKBP5 antagonist treatment may therefore have beneficial psychiatric and physiological outcomes [1]. Although studies investigating peripheral *FKBP5* expression have provided consistent evidence implicating *FKBP5* in psychiatric disorders, less in known about *FKBP5* expression in the brain and further research is needed before human clinical trials involving FKBP5 antagonist treatment can commence [1].

Our finding that rape stigma is a factor in the development and course of PTSD has public health relevance considering that rape stigma is a barrier to disclosure, and receiving legal, psychological and medical intervention, including HIV prophylaxis [158]. This may have downstream individual-level effects such as increased risk for psychiatric disorders, HIV and STIs [158]. It may also have downstream community and society level effects, increasing the likelihood of reoffending by a perpetrator if the perpetrator is not convicted [144]. Reoffending without prosecution may instil the belief that rape is a minor offence and that the victim is to blame for the rape or could have done more to prevent the rape [159].

Rape stigma includes internal thoughts and morally rooted emotions (e.g. self-blaming, shame and embarrassment) as well as actions from significant others (e.g. downplaying the severity of the rape and treating the victim differently) [35,160,161]. Increased rape stigma may therefore result in a decreased ability to access and rely on social support especially when significant other display stigmatising attitudes [35,160,162]. Stigmatising attitudes are especially prevalent when the perpetrator is known to the victim or is a respected member of the community [25,31,35,162,163]. Since rape is a violation at an interpersonal level, it is plausible that victims develop a sense of distrust in others and when this distrust is not repaired over time through positive supportive responses, this may result in more severe emotional distress [22,27].

Women who report fears of being stigmatised following rape are also more likely to have prior sexual traumas and have decreased positive coping mechanisms putting them at increased risk for psychiatric disorders [158]. Decreased positive coping mechanisms and increased negative coping mechanisms such as social isolation and alcohol abuse [162,164,165] may sustain PTSD symptoms and result in an upward PTSD symptom trajectory and chronicity. If rape survivors do report the rape, they have access to a counsellor when at a Thuthuzela Care Centre and this may be an opportunity to deliver a brief intervention targeting stigmatising views of rape [166]. Psychoeducation and brief rape stigma-focussed cognitive behavioural interventions could be delivered by trained counsellors attending to those reporting the rape for the first time [167].

Addressing stigmatising attitudes and gender-based violence on a societal level is more taxing, but programmes based on the ecological systems model do exist and are implemented in South Africa [143,168,169]. These programmes focus on addressing risk factors for gender-based violence which include addressing: inequality between men and women, male sexual entitlement, violence as a means to resolve relationship conflicts, lack of education and employment opportunities for women, hazardous alcohol consumption and a cycle of familial domestic violence [144].

Depression should also be assessed and monitored in rape survivors given that it is a disabling condition and can have significant adverse effects on quality of life including domains related to physical health [170], social interaction [171], parenting [172] and occupational functioning [173]. There is also an increased risk for suicide if a rape victim meets diagnostic criteria for both PTSD and depression [174,175]. Longitudinal monitoring of depression and suicide risk in rape survivors is a less obtainable goal than brief interventions addressing rape stigma, given that it is resource intensive and requires specialised counselling skills [176]. However, there is a growing body of literature providing evidence for the effectiveness of web-based mental health intervention [177,178]. Developing an internet-based intervention for rape survivors targeting depression, PTSD and rape stigma may be an effective tool for improving mental health in a resource constrained setting such as South Africa [86,87]. Internet-based mental health interventions that include symptom monitoring tools may be especially helpful in flagging individuals experiencing high levels of PTSD, depression and high risk for suicide [179] and referring them to counsellors, psychologists or psychiatrists as needed [176].

6.5. RECOMMENDATIONS FOR FUTURE RESEARCH

Our sample consisted of Black African females from a similar socioeconomic background, of similar age and all exposed to rape. While homogeneity of the sample is important when investigating differential methylation in relation to PTSD, it does limit the generalisability of findings. Replicating the findings related to differential methylation in other ethnicity groups, in males and in participants exposed to other trauma types may provide further support for the role of differential methylation of *ADCYAP1* and *BRSK2* in relation to PTSD status and symptom severity.

The diagnostic criteria for PTSD included distinct symptom clusters related to re-experiencing the trauma, avoidance of trauma cues and memories, negative cognitions and hyperarousal [180]. It is plausible that HPA-axis genes (e.g. *ADCYAP1* and *FKBP5*) play a more important role in the hyperarousal symptom cluster compared to other symptom clusters given that hyperarousal includes HPA-axis associated symptoms such as hypervigilance, a heightened startle reaction, difficulty concentrating and difficulty sleeping [180,181]. *BRSK2* methylation may play a more important role in intrusion and avoidance symptoms given that these symptoms may be rooted in altered brain morphology associated with memory functioning [182]. Intrusion and avoidance symptoms include unwanted, upsetting memories,

nightmares, flashbacks and heightened physical reactivity to trauma-related thoughts, feelings and external reminders [180]. We aim to explore differential methylation of *ADCYAP1*, *BRSK2* and *FKBP5* in relation to PTSD symptom clusters in the future.

The RICE parent study collected a vast amount of additional data and biological specimens not mentioned in our study. Additional data gathered included the collection of serum samples at all timepoints. Future work that is being considered includes: (1) investigating PACAP serum levels over time, as increased PACAP blood levels have been associated with increased PTSD symptom severity and an increased acoustic startle reflex response in women compared to men [57,127], and (2) exploring *ADCYAP1* and *ADCYAP1R1* SNPs, especially the rs2267735 SNP of *ADCYAP1R1*, given that it has been associated with decreased *ADCYAP1R1* mRNA expression, increased PTSD symptom severity, increased dark-enhanced startle response and increased amygdala and hippocampal activity in response to viewing threatening face stimuli [57–59,127].

It will be important that other studies investigate *BRSK2*, *ADCYAP1* and *FKBP5* methylation in relation to structural and functional brain changes in rape-exposed women with and without PTSD.

Several biomarkers and indicators of metabolic syndrome were assessed in the RICE study and include: BMI, blood pressure, heart rate, waist circumference, glycated haemoglobin (HbA1c), HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine aminotransferase (ALT), creatine and C reactive protein (CRP). We aim to investigate the relationship between metabolic disease markers and differential methylation of *BRSK2* and *FKBP5* in relation to PTSD in the future, given that *BRSK2* and *FKBP5* are associated with metabolic processes [125,126,183]. We also have access to adiponectin, leptin and resistin genotyping data, methylation data (from our EWAS findings) and serum levels and will investigate the relationship between these adipokines, metabolic syndrome and PTSD in the future.

The relationship between rape stigma and social support in relation to PTSD will also be explored in more detail. Data related to support from the police, legal system, medical professionals, psychological counsellors, family members, friends and intimate partners were collected in the RICE study. We aim to explore the associations between different domains of support and stigmatising thoughts, in relation to PTSD symptom trajectory.

We investigated baseline depression and alcohol use as predictors of PTSD symptom scores over time in this study, but we did not investigate a history of depression and alcohol use as predictors of PTSD scores over time. We will use additional data collected in the RICE

study to determine if depression and alcohol use covaries with PTSD symptoms over time and/or if a history of depression and alcohol use predicts PTSD symptom trajectories. We will also explore the relationship between symptoms of depression and symptoms of PTSD in rape survivors, given that depression was a significant predictor of PTSD scores overtime and that the symptoms of depression potentially overlaps with the symptoms of PTSD.

The age at which childhood trauma occurred and the type of childhood trauma endured may contribute to PTSD risk given heightened brain plasticity in critical periods of developments [14,15]. We will explore the relationship between different childhood trauma types and exposure during different developmental periods as a predictor of PTSD. We will also determine if differential methylation of *BRSK2*, *ADCYAP1* and *FKBP5* mediates the relationship between childhood trauma type/age of exposure and PTSD risk.

Although we did find epigenome-wide significant findings, our EWAS sample was small and replication of the study in a larger sample may implicate additional genes, not identified in our EWAS, in the trajectory of PTSD symptoms. Future studies investigating longitudinal change in methylation in relation to change in PTSD symptoms in larger samples are needed to explore cause and effect relationships further.

REFERENCES

1. Matosin N, Halldorsdottir T, Binder EB. Understanding the Molecular Mechanisms Underpinning Gene by Environment Interactions in Psychiatric Disorders: The FKBP5 Model. *Biol Psychiatry*. 2018;83(10):821-830. doi:10.1016/j.biopsych.2018.01.021

2. Zannas AS, Wiechmann T, Gassen NC, Binder EB. Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications. *Neuropsychopharmacology*. 2016;41(1):261-274. doi:10.1038/npp.2015.235

3. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene – childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41. doi:10.1038/nn.3275

4. Nöthling J, Malan-Müller S, Abrahams N, Joanna Hemmings SM, Seedat S. Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review. *World J Biol Psychiatry*. 2019:1-20. doi:10.1080/15622975.2019.1583369

5. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296

6. Campbell R, Sprague HB, Cottrill S, Sullivan CM. Longitudinal research with sexual assault survivors: A methodological review. *J Interpers Violence*. 2011;26(3):433-461. doi:10.1177/0886260510363424

7. McLaughlin K, Green J, Gruber M, Sampson N, Zaslavsky A, Kessler R. Childhood adversities and adult psychopathology in the National Comorbidity Survey Replication (NCS-R) III: Associations with functional impairment related to DSM-IV disorders. *Psychol Med*. 2010;40(5):847-859. doi:10.1017/S0033291709991115.Childhood

8. Carr CP, Maria ÞC, Martins S, Stingel AM, Lemgruber VB, Juruena MF. The Role of Early Life Stress in Adult Psychiatric Disorders A Systematic Review According to Childhood Trauma Subtypes. *J Nerv Ment Dis*. 2013;201(12):1007-1020. doi:10.1097/NMD.0000000000000049

9. Chen LP, Murad MH, Paras ML, et al. Sexual abuse and lifetime diagnosis of

psychiatric disorders: Systematic review and meta-analysis. *Mayo Clin Proc*. 2010;85(7):618-629. doi:10.4065/mcp.2009.0583

10. Jonas S, Bebbington P, McManus S, et al. Sexual abuse and psychiatric disorder in England : Results from the 2007 Adult Psychiatric Morbidity Survey. *Psychol Med*. 2011:1-11. doi:10.1017/S003329171000111X

11. Afifi TO, Boman J, Fleisher W, Sareen J. Child Abuse & Neglect The relationship between child abuse , parental divorce , and lifetime mental disorders and suicidality in a nationally representative adult sample. *Child Abus Negl*. 2009;33:139-147. doi:10.1016/j.chiabu.2008.12.009

12. Klengel T, Pape J, Binder EB, Mehta D. The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology*. 2014;80:115-132. doi:10.1016/j.neuropharm.2014.01.013

13. Grayson DR, Guidotti A. The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. *Neuropsychopharmacology*. 2013;38(1):138-166. doi:10.1038/npp.2012.125

14. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. *Dev Psychobiol*. 2010;52(7):671-690. doi:10.1002/dev.20494

15. Roth TL, Sweatt JD. Annual research review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. *J Child Psychol Psychiatry Allied Discip*. 2011;52(4):398-408. doi:10.1111/j.1469-7610.2010.02282.x

16. Vinkers CH, Kalafateli AL, Rutten BP, et al. Traumatic stress and human DNA methylation: A critical review. *Epigenomics*. 2015;7(4):593-608. doi:10.2217/epi.15.11

17. Möller AT, Bäckström T, Söndergaard HP, Helström L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One*. 2014;9(10):1-9. doi:10.1371/journal.pone.0111136

18. Meloni M. Race in an epigenetic time: thinking biology in the plural. *Br J Sociol*. 2017;68(3):389-409. doi:10.1111/1468-4446.12248

19. Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci*. 2007;8(5):355-367. doi:10.1038/nrn2132

20. Kubota T, Miyake K, Hirasawa T. Epigenetic understanding of gene-environment interactions in psychiatric disorders: A new concept of clinical genetics. *Specif Gene Expr Epigenetics Interplay Between Genome its Environ*. 2014;4(1):241-258. doi:10.1201/b16680

21. Elklit A, Christiansen DM. ASD and PTSD in rape victims. *J Interpers Violence*. 2010;25(8):1470-1488. doi:10.1177/0886260509354587

22. Elklit A, Christiansen DM. Risk factors for posttraumatic stress disorder in female help-seeking victims of sexual assault. *Violence Vict*. 2013;28(3):552-568. doi:10.1891/0886-6708.09-135

23. Nickerson A, Steenkamp M, Aerka IM, et al. Prospective investigation of mental health following sexual assault. *Depress Anxiety*. 2013;30(5):444-450. doi:10.1002/da.22023

24. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

25. Ullman S, Filipas H. Predictors of PTSD symptom severity and social reactions in sexual assault victims. *J Trauma Stress*. 2001;14(2):369-389. doi:10.1023/A

26. Ullman S, Filipas H, Townsend S, Starzynski L. Psychosocial correlates of PTSD symptom severity in sexual assault survivors. *J Trauma Stress*. 2007;20(5):821-831. doi:10.1002/jts.20290

27. Bryant-Davis T, Ullman SE, Tsong Y, Tillman S, Smith K. Struggling to survive: Sexual assault, poverty, and mental health outcomes of african american women. *Am J Orthopsychiatry*. 2010;80(1):61-70. doi:10.1111/j.1939-0025.2010.01007.x

28. Nishith P, Mechanic MB, Resick PA. Prior interpersonal trauma: The contribution to current PTSD symptoms in female rape victims. *J Abnorm Psychol*. 2000;109(1):20-25. doi:10.1037/0021-843X.109.1.20

29. Machtinger EL, Wilson TC, Haberer JE, Weiss DS. Psychological trauma and PTSD in HIV-positive women: A meta-analysis. *AIDS Behav*. 2012;16(8):2091-2100.

doi:10.1007/s10461-011-0127-4

30. Kaysen D, Rosen G, Bowman M, Resick PA. Duration of Exposure and the Dose-Response Model of PTSD. *J Interpers Violence*. 2010;25(1):63-74. doi:10.1177/0886260508329131

31. Ullman SE, Filipas HH, Townsend SM, Starzynski LL. The role of victim-offender relationship in women's sexual assault experiences. *J Interpers Violence*. 2006;21(6):798-819. doi:10.1177/0886260506288590

32. Bryant-Davis T, Ullman SE, Tsong Y, Gobin R. Surviving the storm: The role of social support and religious coping in sexual assault recovery of African American women. *Violence Against Women*. 2011;17(12):1601-1618. doi:10.1177/1077801211436138

33. Kessler R, Rose S, Koenen K, et al. How well can post-traumatic stress disorder be predicted from pre-trauma risk factors ? An exploratory study in the WHO World Mental Health Surveys. *World Psychiatry*. 2014;13:265-274.

34. Kleber R. Trauma and Public Mental Health : A Focused Review. *Front Psychiatry*. 2019;10:1-6. doi:10.3389/fpsyt.2019.00451

35. Moor A, Farchi M. Is rape-related self blame distinct from other post traumatic attributions of blame? A comparison of severity and implications for treatment. *Women Ther*. 2011;34(4):447-460. doi:10.1080/02703149.2011.591671

36. Breslau N, Davis GC, Peterson EL, Schultz LR. A second look at comorbidity in victims of trauma: The posttraumatic stress disorder-major depression connection. *Biol Psychiatry*. 2000;48(9):902-909. doi:10.1016/S0006-3223(00)00933-1

37. Resnick HS, Walsh K, Schumacher JA, Kilpatrick DG, Acierno R. Prior substance abuse and related treatment history reported by recent victims of sexual assault. *Addict Behav*. 2013;38(4):2074-2079. doi:10.1016/j.addbeh.2012.12.010

38. Resnick HS, Walsh K, McCauley JL, Schumacher JA, Kilpatrick DG, Acierno RE. Assault related substance use as a predictor of substance use over time within a sample of recent victims of sexual assault. *Addict Behav*. 2012;37(8):914-921. doi:10.1016/j.addbeh.2012.03.017

39. Testa M, Livingston JA, Hoffman JH. Does sexual victimization predict subsequent

alcohol consumption? A prospective study among a community sample of women. *Addict Behav*. 2007;32(12):2926-2939. doi:10.1016/j.addbeh.2007.05.017

40. Kaysen D, Neighbors C, Martell J, Fossos N, Larimer ME. Incapacitated rape and alcohol use: A prospective analysis. *Addict Behav*. 2006;31(10):1820-1832. doi:10.1016/j.addbeh.2005.12.025

41. Kilpatrick DG, Ruggiero KJ, Acierno R, Saunders BE, Resnick HS, Best CL. Violence and risk of PTSD, major depression, substance abuse/dependence, and comorbidity: Results from the national survey of adolescents. *J Consult Clin Psychol*. 2003;71(4):692-700. doi:10.1037/0022-006X.71.4.692

42. Santiago PN, Ursano RJ, Gray CL, et al. A Systematic Review of PTSD Prevalence and Trajectories in DSM-5 Defined Trauma Exposed Populations : Intentional and Non-Intentional Traumatic Events. *PLoS One*. 2013;8(4):e59236. doi:10.1371/journal.pone.0059236

43. Mgoqi-Mbalo N, Zhang M, Ntuli S. Risk factors for PTSD and depression in female survivors of rape. *Psychol Trauma Theory, Res Pract Policy*. 2017;9(3):301-308. doi:10.1037/tra0000228

44. Ryan J, Chaudieu I, Saffery R. Biological underpinnings of trauma and post-traumatic stress disorder: focusing on genetics and epigenetics. *Epigenomics*. 2016;8(11):1553-1569.

45. Nievergelt CM, Ashley-Koch AE, Dalvie S, et al. Genomic Approaches to Posttraumatic Stress Disorder: The Psychiatric Genomic Consortium Initiative. *Biol Psychiatry*. 2018;83(10):831-839. doi:10.1016/j.biopsych.2018.01.020

46. Daskalakis NP, Rijal CM, King C, Huckins LM, Ressler KJ. Recent Genetics and Epigenetics Approaches to PTSD. *Curr Psychiatry Rep*. 2018;20(5). doi:10.1007/s11920-018-0898-7

47. Morrison FG, Miller MW, Logue MW, Assef M, Wolf EJ. DNA methylation correlates of PTSD: Recent findings and technical challenges. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2019;90(November 2018):223-234. doi:10.1016/j.pnpbp.2018.11.011

48. Mehta D, Klengel T, Conneely KN, et al. Childhood maltreatment is associated with

distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2013;110(20):8302-8307. doi:10.1073/pnas.1217750110

49. Hammamieh R, Chakraborty N, Gautam A, et al. Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Transl Psychiatry*. 2017;7(7):e1169. doi:10.1038/tp.2017.129

50. Smith A, Conneely K, Kilaru V, et al. Differential immune system DNA methylation and cytokine regulation in Post-Traumatic Stress Disorder. *Am J Med Genet Paert B Neuropsychiatr Genet*. 2011;156B(6):700-708. doi:10.1109/TMI.2012.2196707.Separate

51. Mehta D, Bruenig D, Carrillo-Roa T, et al. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatr Scand*. 2017;136(5):493-505. doi:10.1111/acps.12778

52. Rutten BPF, Vermetten E, Vinkers CH, et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry*. 2018;23(5):1145-1156. doi:10.1038/mp.2017.120

53. Snijders C, Maihofer AX, Ratanatharathorn A, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clin Epigenetics*. 2020;12(1):1-13. doi:10.1186/s13148-019-0798-7

54. Maddox SA, Kilaru V, Shin J, et al. Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. *Mol Psychiatry*. 2018;23(3):658-665. doi:10.1038/mp.2016.250

55. Uddin M, Aiello AE, Wildman DE, et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2010;107(20):9470-9475. doi:10.1073/pnas.0910794107

56. Chen Y, Li X, Kobayashi I, Tsao D, Mellman T. Expression and methylation in Posttraumatic Stress Disorder and resilience; evidence of the role of odorant receptors. *Psychiatry Res*. 2016;245:36-44. doi:10.1016/j.physbeh.2017.03.040

57. Ressler K, Mercer K, Bradley B, et al. Post-traumatic stress disorder is associated with

PACAP and PAC1 receptor. *Nature*. 2011;470(7335):492-497. doi:10.1097/CCM.0b013e31823da96d.Hydrogen

58. Almli LM, Mercer KB, Kerley K, et al. ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. *Am J Med Genet Part B Neuropsychiatr Genet*. 2013;162(3):262-272. doi:10.1002/ajmg.b.32145

59. Stevens JS, Almli LM, Fani N, et al. PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. *Proc Natl Acad Sci U S A*. 2014;111(8):3158-3163. doi:10.1073/pnas.1318954111

60. Edmondson D, Kronish IM, Shaffer JA, Falzon L, Burg MM. Posttraumatic stress disorder and risk for coronary heart disease: A meta-analytic review. *Am Heart J*. 2013;166(5):806-814. doi:10.1016/j.ahj.2013.07.031

61. Ryder AL, Azcarate PM, Cohen BE. PTSD and Physical Health. *Curr Psychiatry Rep*. 2018;20(12). doi:10.1007/s11920-018-0977-9

62. Mustafa T. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP). *Adv Pharmacol*. 2013;68:445-457. doi:10.1016/B978-0-12-411512-5.00021-X

63. Rudecki AP, Gray SL. PACAP in the Defense of Energy Homeostasis. *Trends Endocrinol Metab*. 2016;27(9):620-632. doi:10.1016/j.tem.2016.04.008

64. Hammack SE, Cheung J, Rhodes KM, et al. Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): Roles for PACAP in anxiety-like behavior. *Psychoneuroendocrinology*. 2009;34(6):833-843. doi:10.1016/j.psyneuen.2008.12.013

65. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*. 2009;34(SUPPL. 1):186-195. doi:10.1016/j.psyneuen.2009.05.021

66. Smoller JW. The Genetics of Stress-Related Disorders: PTSD, Depression, and Anxiety Disorders. *Neuropsychopharmacology*. 2016;41(1):297-319. doi:10.1038/npp.2015.266

67. Wang Q, Shelton RC, Dwivedi Y. Interaction between early-life stress and FKBP5

gene variants in major depressive disorder and post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2018;225(July 2017):422-428. doi:10.1016/j.jad.2017.08.066

68. Yehuda R, Daskalakis NP, Bierer LM, et al. Archival Report Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. *Biol Psychiatry*. 2016;80(5):372-380. doi:10.1016/j.biopsych.2015.08.005

69. Ramo-fernández L, Boeck C, Koenig AM, et al. The effects of childhood maltreatment on epigenetic regulation of stress-response associated genes : an intergenerational approach. *Sci Rep*. 2019;9(983):1-12. doi:10.1038/s41598-018-36689-2

70. Bishop JR, Lee AM, Mills LJ, et al. Methylation of FKBP5 and SLC6A4 in Relation to Treatment Response to Mindfulness Based Stress Reduction for Posttraumatic Stress Disorder. *Front Psychiatry*. 2018;9(September):1-11. doi:10.3389/fpsyt.2018.00418

71. Kang J, Kim T, Choi J, So H, Kim S. Allele-specific DNA methylation level of FKBP5 is associated with post- traumatic stress disorder. *Psychoneuroendocrinology*. 2019;103(November 2018):1-7. doi:10.1016/j.psyneuen.2018.12.226

72. Taft CT, Resick PA, Watkins LE, Panuzio J. An investigation of posttraumatic stress disorder and depressive symptomatology among female victimsof interpersonal trauma. *J Fam Violence*. 2009;24(6):407-415. doi:10.1007/s10896-009-9243-6

73. Nixon RD V, Resick PA, Nishith P. An exploration of comorbid depression among female victims of intimate partner violence with posttraumatic stress disorder. *J Affect Disord*. 2004;82(2):315-320. doi:10.1016/j.jad.2004.01.008

74. Szyf M. How do environments talk to genes? *Nat Neurosci*. 2013;16(1):1-4.

75. Jaaskelainen T, Makkonen H, Palvimo J. Steroid up-regulation of FKBP51 and its role in hormone signaling. *Curr Opin Pharmacol*. 2011;11:326-331. doi:10.1016/j.coph.2011.04.006

76. Daskalakis NP, Mcgill MA, Lehrner A, Yehuda R. Endocrine aspects of PTSD: hypothalamic-pituitary-adrenal (HPA) axis and beyond. In: Martin C, Preedy V, Patel V, eds. *Comprehensive Guide to Post-Traumatic Stress Disorders*. Switzerland: Springer International Publishing; 2015. doi:10.1007/978-3-319-08359-9

77. de Kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, Westenberg HGM. Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. *J Psychiatr Res*. 2006;40(6):550-567. doi:10.1016/j.jpsychires.2005.08.002

78. McGowan PO, Sasaki A, D'Alessio AC, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci*. 2009;12(3):342-348. doi:10.1038/nn.2270

79. Perroud N, Paoloni-Giacobino A, Prada P, et al. Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl Psychiatry*. 2011;1(12):e59. doi:10.1038/tp.2011.60

80. Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. *PLoS One*. 2012;7(1). doi:10.1371/journal.pone.0030148

81. Melas PA, Wei Y, Wong CCY, et al. Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *Int J Neuropsychopharmacol*. 2013;16(7):1513-1528. doi:10.1017/S1461145713000102

82. Schechter DS, Moser DA, Paoloni-Giacobino A, et al. Methylation of NR3C1 is related to maternal PTSD, parenting stress and maternal medial prefrontal cortical activity in response to child separation among mothers with histories of violence exposure. *Front Psychol*. 2015;6(May):1-12. doi:10.3389/fpsyg.2015.00690

83. Bustamante AC, Aiello AE, Galea S, et al. Glucocorticoid receptor DNA methylation, childhood maltreatment and major depression. *J Affect Disord*. 2016;206:181-188. doi:10.1016/j.jad.2016.07.038

84. Shields AE, Wise LA, Ruiz-Narvaez EA, et al. Childhood abuse, promoter methylation of leukocyte NR3C1 and the potential modifying effect of emotional support. *Epigenomics*. 2016;8(11):1507-1517. doi:10.2217/epi-2016-0074

85. Wang W, Feng J, Ji C, et al. Increased methylation of glucocorticoid receptor gene promoter 1F in peripheral blood of patients with generalized anxiety disorder. *J Psychiatr Res*. 2017;91:18-25. doi:10.1016/j.jpsychires.2017.01.019

86. Kearns M, Ressler K, Zatzick D, Rothbaum B. Early interventions for ptsd: a review. *Depress Anxiety*. 2012;842:833-842. doi:10.1002/da.21997

87. Tol WA, Barbui C, Bisson J, et al. World Health Organization Guidelines for Management of Acute Stress , PTSD , and Bereavement : Key Challenges on the Road Ahead. *PLOS Med*. 2014;11(12):e1001769. doi:10.1371/journal.pmed.1001769

88. Bruckner TA, Scheffler RM, Shen G, et al. The mental health workforce gap in low- and middle-income countries: A needs-based approach. *Bull World Health Organ*. 2011;89(3):184-194. doi:10.2471/BLT.10.082784

89. Uddin M, Ratanatharathorn A, Armstrong D, Kuan P, Allison E. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics*. 2018;10(12):1585-1601.

90. Smith A, Ratanatharathorn A, Maihofer A, et al. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies novel methylation loci. *bioRxiv Prepr*. 2019. doi:https://doi.org/10.1101/585109

91. Evans GW, Kantrowitz E. Socioeconomic Status and Health: The Potential Role of Environmental Risk Exposure. *Annu Rev Public Health*. 2002;23(1):303-331. doi:10.1146/annurev.publhealth.23.112001.112349

92. Galanter JM, Gignoux CR, Oh SS, et al. Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. *Elife*. 2017;6:e20532. doi:10.7554/eLife.20532

93. Xia Y yin, Ding Y bing, Liu X qing, et al. Racial/ethnic disparities in human DNA methylation. *Biochim Biophys Acta*. 2014;1846:258-262. doi:10.1016/j.bbcan.2014.07.001

94. Breslau N, Troost JP, Bohnert K, Luo Z. Influence of predispositions on post-traumatic stress disorder: Does it vary by trauma severity? *Psychol Med*. 2013;43(2):381-390. doi:10.1017/S0033291712001195

95. Yehuda R, Mcfarlane A, Vermetten E, Lanius R. Post-traumatic stress disorder. *Nat Rev*. 2015;1(October):1-22. doi:10.1038/nrdp.2015.57

96. Merikangas AK, Almasy L. Using the tools of genetic epidemiology to understand sex differences in neuropsychiatric disorders. *Genes, Brain Behav*. 2020;19:e12660.

doi:10.1111/gbb.12660

97. Chung WCJ, Auger AP. Gender differences in neurodevelopment and epigenetics. *Eur J Physiol*. 2013;465(5):573-584. doi:10.1007/s00424-013-1258-4

98. Uddin M, Sipahi L, Li J, Koenen KC. Sex differences in dna methylation may contribute to risk of PTSD and depression: A review of existing evidence. *Depress Anxiety*. 2013;30(12):1151-1160. doi:10.1002/da.22167

99. Rakyan V, Down T, Maslau S, et al. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res*. 2010;20(4):434–9. doi:10.1101/gr.103101.109.434

100. Hernandez DG, Nalls MA, Gibbs JR, et al. Distinct DNA methylation changes highly correlated with chronological age in the human brain. *Hum Mol Genet*. 2011;20(6):1164-1172. doi:10.1093/hmg/ddq561

101. Teschendorff AE, Menon U, Gentry-Maharaj A, et al. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res*. 2010;20(4):440-446. doi:10.1101/gr.103606.109

102. Bell JT, Tsai PC, Yang TP, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet*. 2012;8(4):e1002629. doi:10.1371/journal.pgen.1002629

103. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CPG island context. *PLoS Genet*. 2009;5(8):e1000602. doi:10.1371/journal.pgen.1000602

104. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3

105. Koukoura O, Sifakis S, Spandidos DA. DNA methylation in the human placenta and fetal growth (review). *Mol Med Rep*. 2012;5(4):883-889. doi:10.3892/mmr.2012.763

106. Braun PR, Han S, Hing B, et al. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. *Transl Psychiatry*. 2019;9(1). doi:10.1038/s41398-019-0376-y

107. Ratanatharathorn A, Aiello A, Boks M, et al. Epigenetic Biomarkers Of PTSD:

Updates From The EWAS Working Group of The PTSD PGC. *Eur Neuropsychopharmacol*. 2019;29:S750. doi:10.1016/j.euroneuro.2017.06.092

108. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13(1). doi:10.1186/1471-2105-13-86

109. Adalsteinsson BT, Gudnason H, Aspelund T, et al. Heterogeneity in White Blood Cells Has Potential to Confound DNA Methylation Measurements. *PLoS One*. 2012;7(10):e46705. doi:10.1371/journal.pone.0046705

110. Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cells: Implications for cell lineage and studies on disease susceptibility. *PLoS One*. 2012;7:e41361. doi:10.1371/journal.pone.0041361

111. Csoka AB, Szyf M. Epigenetic side-effects of common pharmaceuticals: A potential new field in medicine and pharmacology. *Med Hypotheses*. 2009;73:770-780. doi:10.1016/j.mehy.2008.10.039

112. Zhang R, Miao Q, Wang C, et al. Genome-wide DNA methylation analysis in alcohol dependence. *Addict Biol*. 2013;18(2):392-403. doi:10.1111/adb.12037

113. Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. *Alcohol Res Curr Rev*. 2012;35(1):25-35.

114. Lee KWK, Pausova Z. Cigarette smoking and DNA methylation. *Front Genet*. 2013;4(JUL):1-11. doi:10.3389/fgene.2013.00132

115. Gornalusse GG, Mummidi S, Gaitan AA, et al. Epigenetic mechanisms, T-cell activation, and CCR5 genetics interact to regulate T-cell expression of CCR5, the major HIV-1 coreceptor. *Proc Natl Acad Sci U S A*. 2015;112(34):E4762-E4771. doi:10.1073/pnas.1423228112

116. Nakayama-Hosoya K, Ishida T, Youngblood B, et al. Epigenetic repression of interleukin 2 expression in senescent CD4+ T cells during chronic HIV type 1 infection. *J Infect Dis*. 2015;211(1):28-39. doi:10.1093/infdis/jiu376

117. Zhang Y, Li SK, Yi Yang K, et al. Whole genome methylation array reveals the down-regulation of IGFBP6 and SATB2 by HIV-1. *Sci Rep*. 2015;5:1-14. doi:10.1038/srep10806

118. Nelson KN, Hui Q, Rimland D, et al. Identification of HIV infection-related DNA methylation sites and advanced epigenetic aging in HIV-positive, treatment-naive U.S. veterans. *Aids*. 2017;31(4):571-575. doi:10.1097/QAD.0000000000001360

119. Rosenbaum S, Stubbs B, Ward PB, Steel Z, Lederman O, Vancampfort D. The prevalence and risk of metabolic syndrome and its components among people with posttraumatic stress disorder: A systematic review and meta-analysis. *Metabolism*. 2015;64(8):926-933. doi:10.1016/j.metabol.2015.04.009

120. Michopoulos V, Vester A, Neigh G. Posttraumatic stress disorder: A metabolic disorder in disguise? *Exp Neurol*. 2016;284:220-229. doi:10.1016/j.expneurol.2016.05.038

121. Barres R, Zierath JR. DNA methylation in metabolic disorders. *Am J Clin Nutr*. 2011;93(4):897-900. doi:10.3945/ajcn.110.001933

122. Danese A, Moffitt T, Pariante C, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Arch Gen Psychiatry*. 2008;65(4):409-416. doi:10.1001/archpsyc.65.6.725

123. Danese A, Moffitt TE, Harrington HL, et al. Adverse childhood experiences and adult risk factors for age-related disease. *Arch Pediatr Adolesc Med*. 2009;163(12):1135-1143. doi:10.1001/archpediatrics.2009.214

124. Suderman M, Borghol N, Pappas JJ, et al. Childhood abuse is associated with methylation of multiple loci in adult DNA. *BMC Med Genomics*. 2014;7(1). doi:10.1186/1755-8794-7-13

125. Bright NJ, Carling D, Thornton C. Investigating the Regulation of Brain-specific Kinases 1 and 2 by Phosphorylation *. 2008;283(22):14946-14954. doi:10.1074/jbc.M710381200

126. Chen X, Gu X, Saiyin H, et al. Brain-selective Kinase 2 (BRSK2) Phosphorylation on PCTAIRE1 Negatively Regulates Glucose-stimulated Insulin Secretion in Pancreatic □ -Cells * □. 2012;287(36):30368-30375. doi:10.1074/jbc.M112.375618

127. Mcmahon FJ, Akula N, Schulze TG, et al. Meta-analysis of genome-wide association data detects a risk locus for major mood disorders on chromosome 3p21.1. *Genome*. 2010;42(2):128-131. doi:10.1038/ng.523.Meta-analysis

128. Binder EB, Salyakina D, Lichtner P, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet*. 2004;36(12):1319-1325. doi:10.1038/ng1479

129. Binder EB, Bradley RG, Epstein MP, et al. Association of FKBP5 Polymorphisms and Childhood Abuse With Risk of Posttraumatic Stress Disorder Symptoms in Adults. *JAMA*. 2008;299(11):1291-1305.

130. Post LM, Zoellner LA, Youngstrom E, Feeny NC. Understanding the relationship between co-occurring PTSD and MDD: Symptom severity and affect. *J Anxiety Disord*. 2011;25(8):1123-1130. doi:10.1016/j.janxdis.2011.08.003

131. Flory J, Yehuda R. Comorbidity between post-traumatic stress disorder and major depressive disorder: alternative explanations and treatment considerations. *Dialogues Clin Neurosci*. 2015;17(2):141-150.

132. Spinhoven P, Penninx BW, Hemert AM Van, Rooij M De, Elzinga BM. Child Abuse & Neglect Comorbidity of PTSD in anxiety and depressive disorders : Prevalence and shared risk factors &. *Child Abuse Negl*. 2014;38(8):1320-1330. doi:10.1016/j.chiabu.2014.01.017

133. Greene T, Neria Y, Gross R. Prevalence , Detection and Correlates of PTSD in the Primary Care Setting : A Systematic Review. *J Clin Psychol Med Settings*. 2016;23(2):160-180. doi:10.1007/s10880-016-9449-8

134. Simpson TL, Miller WR. Concomitance between childhood sexual and physical abuse and substance use problems: A review. *Clin Psychol Rev*. 2002;22(1):27-77. doi:10.1016/S0272-7358(00)00088-X

135. Logan T, Walker R, Cole J, Leukefeld C. Victimization and Substance Abuse among Women: Contributing Factors, Interventions, and Implications. *Rev Gen Psychol*. 2002;6(4):325-397. doi:10.1037/1089-2680.6.4.325

136. Mill J, Heijmans BT. From promises to practical strategies in epigenetic epidemiology. *Nat Rev Genet*. 2013;14(8):585-594. doi:10.1038/nrg3405

137. Rollins B, Martin M V., Morgan L, Vawter MP. Analysis of whole genome biomarker expression in blood and brain. *Am J Med Genet Part B Neuropsychiatr Genet*. 2010;153(4):919-936. doi:10.1002/ajmg.b.31062

138. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet - Neuropsychiatr Genet*. 2006;141 B(3):261-268. doi:10.1002/ajmg.b.30272

139. Fortin J, Labbe A, Lemire M, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol*. 2014;15(503):1-17.

140. Boals A. Trauma in the eye of the beholder: Objective and subjective definitions of trauma. *J Psychother Integr*. 2018;28(1):77-89. doi:10.1037/int0000050

141. Dancu C V., Riggs DS, Hearst-Ikeda D, Shoyer BG, Foa EB. Dissociative experiences and posttraumatic stress disorder among female victims of criminal assault and rape. *J Trauma Stress*. 1996;9(2):253-267. doi:10.1007/BF02110659

142. McCart M, Smith D, Sawyer G. Help seeking among victims of crime: A review of the epirical literature. *J Trauma Stress*. 2010;23(2):198-206. doi:10.1002/jts

143. Jewkes R, Sikweyiya Y, Morrell R, Dunkle K. Gender inequitable masculinity and sexual entitlement in rape perpetration south africa: Findings of a cross-sectional study. *PLoS One*. 2011;6(12). doi:10.1371/journal.pone.0029590

144. Reconciliation Centre for the Study of Violence and Reconciliation. Evidence-based strategies for the prevention of gender-based violence in South Africa: A case study of CSVR. 2016;(April):1-18.

145. Mehta D, Miller O, Bruenig D, David G, Shakespeare-Finch J. A Systematic Review of DNA Methylation and Gene Expression Studies in Posttraumatic Stress Disorder, Posttraumatic Growth, and Resilience. *J Trauma Stress*. 2020;33(2):171-180. doi:10.1002/jts.22472

146. Dias BG, Ressler KJ. PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology*. 2013;38(1):245-247. doi:10.1038/npp.2012.147

147. Mercer KB, Dias B, Shafer D, et al. Functional evaluation of a PTSD-associated genetic variant : estradiol regulation and ADCYAP1R1. 2016;(August):1-7. doi:10.1038/tp.2016.241

148. Kalsi G, Euesden J, Coleman JRI, et al. Genome-Wide Association of Heroin Dependence in Han Chinese. 2016:1-18. doi:10.1371/journal.pone.0167388

149. Tamir T, Bowman B, Agajanian M, et al. Gain-of-function genetic screen of the kinome reveals BRSK2 as an inhibitor of the NRF2 transcription factor. *J Cell Sci*. 2020;133:jcs241356. doi:10.13861/j.cnki.sece.2019.05.003

150. Kim JSM, Lilley BN, Zhang C, Shokat KM, Sanes JR, Zhen M. A chemical-genetic strategy reveals distinct temporal requirements for SAD-1 kinase in neuronal polarization and synapse formation. *Neural Dev*. 2008;3(23):1-14. doi:10.1186/1749-8104-3-23

151. Kishi M, Pan YA, Crump JG, Sanes JR. Mammalian SAD kinases are required for neuronal polarization. *Science (80-)*. 2005;307(5711):929-932. doi:10.1126/science.1107403

152. Sample V, Ramamurthy S, Gorshkov K, Ronnett G V, Chernoff J. Polarized activities of AMPK and BRSK in primary hippocampal neurons. 2015;26:1935-1946. doi:10.1091/mbc.E14-02-0764

153. Inoue E, Mochida S, Takagi H, et al. SAD: A Presynaptic Kinase Associated with Synaptic Vesicles and the Active Zone Cytomatrix that Regulates Neurotransmitter Release. *Neuron*. 2006;50(2):261-275. doi:10.1016/j.neuron.2006.03.018

154. Kelmendi B, Adams TG, Yarnell S, Southwick S, Abdallah CG, Krystal JH. PTSD: from neurobiology to pharmacological treatments. *Eur J Psychotraumatol*. 2016;7(1):31858. doi:10.3402/ejpt.v7.31858

155. Gaali S, Kirschner A, Cuboni S, et al. Selective inhibitors of the FK506-binding protein 51 by induced fit. *Nat Chem Biol*. 2015;11(1):33-37. doi:10.1038/nchembio.1699

156. Hartmann J, Wagner K V., Gaali S, et al. Pharmacological inhibition of the psychiatric risk factor FKBP51 has anxiolytic properties. *J Neurosci*. 2015;35(24):9007-9016. doi:10.1523/JNEUROSCI.4024-14.2015

157. Balsevich G, Häusl AS, Meyer CW, et al. Stress-responsive FKBP51 regulates AKT2-AS160 signaling and metabolic function. *Nat Commun*. 2017;8(1). doi:10.1038/s41467-017-01783-y

158. Abrahams N, Jewkes R. Barriers to post exposure prophylaxis (PEP) completion after rape: A South African qualitative study. *Cult Heal Sex*. 2010;12(5):471-484.

doi:10.1080/13691050903556316

159. Kasparian A. Justice beyond bars: exploring the restorative justice alternative for victims of rape and sexual assault. *Suffolk Transnatl Law R*. 2014;37(2):377-409.

160. Carretta CM, Burgess AW, DeMarco R. To tell or not to tell. [References]. *Violence Against Women*. 2016;21(9):1145-1165. doi:10.1177/1077801215590672

161. Starzynski LL, Ullman SE, Filipas HH, Townsend SM. Correlates of women's sexual assault disclosure to informal and formal support sources. *Violence Vict*. 2005;20(4):417-432. doi:10.1891/0886-6708.20.4.417

162. Wasco SM. Conceptualizing the Harm done by Rape: Applications of Trauma Theory to Experiences of Sexual Assault. *Trauma, Violence, Abus*. 2003;4(4):309-322. doi:10.1177/1524838003256560

163. Carretta CM, Burgess AW, DeMarco R. To Tell or Not to Tell. *Violence Against Women*. 2016;22(13):1499-1518. doi:10.1177/1077801215627507

164. Lilly MM, London MJ, Bridgett DJ. Using SEM to examine emotion regulation and revictimization in predicting PTSD symptoms among childhood abuse survivors. *Psychol Trauma Theory, Res Pract Policy*. 2014;6(6):644-651. doi:10.1037/a0036460

165. Weich S, Patterson J, Shaw R, Stewart-Brown S. Family relationships in childhood and common psychiatric disorders in later life: systematic review of prospective studies. *Br J Psychiatry*. 2009;194(5):392-398. doi:10.1192/bjp.bp.107.042515

166. South African Government. Thuthuzela Care Centres. https://www.gov.za/TCC. Accessed November 13, 2019.

167. Lomax J, Meyrick J. Systematic Review: Effectiveness of psychosocial interventions on wellbeing outcomes for adolescent or adult victim/survivors of recent rape or sexual assault. *J Health Psychol*. 2020. doi:10.1177/1359105320950799

168. Chirwa ED, Sikweyiya Y, Addo-Lartey AA, et al. Prevalence and risk factors of physical or sexual intimate violence perpetration amongst men in four districts in the central region of Ghana: Baseline findings from a cluster randomised controlled trial. *PLoS One*. 2018;13(3):1-18. doi:10.1371/journal.pone.0191663

169. Machisa M, Jewkes R, Morna C, Rama K. *The War at Home*. Johannesburg, South

Africa; 2011.

170. Scott D, Hons BHM, Happell B. The High Prevalence of Poor Physical Health and Unhealthy Lifestyle Behaviours in Individuals with Severe Mental Illness. *Issues Ment Health Nurs*. 2011;32:589-597. doi:10.3109/01612840.2011.569846

171. Orchowski LM, Untied AS, Gidycz CA. Social Reactions to Disclosure of Sexual Victimization and Adjustment Among Survivors of Sexual Assault. *J Interpers Violence*. 2013;28(10):2005-2023. doi:10.1177/0886260512471085

172. Mitchell SJ, Lewin A, Horn IB, Valentine D, Sanders-phillips K, Joseph JG. Social Science & Medicine How does violence exposure affect the psychological health and parenting of young African-American mothers ? *Soc Sci Med*. 2010;70(4):526-533. doi:10.1016/j.socscimed.2009.10.048

173. Hanson RF, Sawyer GK, Begle AM, Hubel GS. The Impact of Crime Victimization on Quality of Life. *J Trauma Stress*. 2010;23(2):189-197. doi:10.1002/jts.

174. Krysinska K, Lester D, Krysinska K, Lester D. Post-Traumatic Stress Disorder and Suicide Risk : A Systematic Review Post-Traumatic Stress Disorder and Suicide Risk : A Systematic Review. *Arch Suicide Res*. 2010;1118(14):1-23. doi:10.1080/13811110903478997

175. Brooker C, Tocque K. Journal of Forensic and Legal Medicine Mental health risk factors in sexual assault : What should Sexual Assault Referral Centre staff be aware of ? *J Forensic Leg Med*. 2016;40:28-33. doi:10.1016/j.jflm.2016.01.028

176. Franx G, Oud M, de Lange J, Wensing M, Grol R. Implementing a stepped-care approach in primary care: Results of a qualitative study. *Implement Sci*. 2012;7(1):1-13. doi:10.1186/1748-5908-7-8

177. Andersson G, Carlbring P, Titov N, Lindefors N. Internet Interventions for Adults with Anxiety and Mood Disorders : A Narrative Umbrella Review of Recent Meta-Analyses. *Can J Psychiatry*. 2019;64(7):465-470. doi:10.1177/0706743719839381

178. Simblett S, Cantab PD, Birch J, et al. A Systematic Review and Meta-Analysis of e-Mental Health Interventions to Treat Symptoms of Posttraumatic Stress Corresponding Author : *JMIR Ment Heal*. 2017;4(2):e14. doi:10.2196/mental.5558

179. Decker V, Valenti M, Montoya V, Sikorskii A, Given CW, Given BA. Maximizing

New Technologies to Treat Depression. *Issues Ment Health Nurs*. 2019;40(3):200-207. doi:10.1080/01612840.2018.1527422

180. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, USA; 2013.

181. Watkins LE, Han S, Harpaz-rotem I, et al. Psychoneuroendocrinology FKBP5 polymorphisms , childhood abuse , and PTSD symptoms : Results from the National Health and Resilience in Veterans Study. *Psychoneuroendocrinology*. 2016;69:98-105. doi:10.1016/j.psyneuen.2016.04.001

182. Thomaes K, Dorrepaal E, Draijer N, Jansma EP, Veltman DJ, van Balkom AJ. Can pharmacological and psychological treatment change brain structure and function in PTSD? A systematic review. *J Psychiatr Res*. 2014;50(1):1-15. doi:10.1016/j.jpsychires.2013.11.002

183. Gaete HP. Hypothalamus-pituitary-adrenal (HPA) axis , chronic stress , hair cortisol , metabolic syndrome and mindfulness. *Integr Mol Med*. 2016;3(5):776-779. doi:10.15761/IMM.1000244

SUPPLEMENTARY MATERIAL

CHAPTER 3: SUPPLEMENTARY MATERIAL

Supplementary Table 1: *Difference is baseline pre- and post-assault characteristics for those who completed all visits vs those who missed one or more visit/s*

	Gorup1: Completed all visits			Group 2: Intermittent attendance			Group 3: Baseline only			*p*
	n	%	M(SD)	n	%	M(SD)	n	%	M(SD)	
PTSD (DTS)	274	100	67.8(25.4)	140	100	67.3(25.4)	225	100	68.9(23.4)	.774
Age	274	100	25.4(5.6)	140	100	24.4(5.1)	225	100	24.2(4.8)	.091
Basic education completed	274	100		140	100		225	100		.616
No	118	43.1		63	45.0		90	40		
Yes	156	56.9		77	55.0		135	60		
Relationship status	274	100		140	100		225	100		.287
In a relationship	214	78.1		111	79.3		191	84.9		
Widowed/separated/divorced	1	0.4		0	0.0		0	0.0		
Single	59	21.5		29	20.7		34	15.1		
Employment status	274	100		140	100		225	100		.531
Employed	64	23.4		26	18.6		48	21.3		
Unemployed	210	76.6		114	81.4		177	78.7		
HIV status	274	100		140	100		225	100		.853
Positive	126	46.0		68	48.6		103	45.8		
Negative	148	54.0		72	51.4		122	54.2		
Childhood trauma (CTQ-SF)[1]	274	100	16.6(3.3)	140	100	15.8(2.5)	225	100	16.4(4.1)	.026*
Number of childhood traumas (CTQ-SF)[1]	274	100		140	100		225	100		.059
No childhood trauma	93	34.0		62	44.3		89	39.6		
1 childhood trauma	59	21.5		39	27.9		57	25.3		
2 childhood traumas	63	23.0		20	14.3		38	16.9		
3 or more childhood traumas	59	21.5		19	13.6		41	18.2		
Lifetime cumulative trauma (LEC)[1]	274	100	2.4(1.5)	140	100	2.5(1.6)	225	100	2.2(1.4)	.328

Number of lifetime traumas (LEC)[1]	274	100		140	100		225	100		.237
No lifetime traumas	54	19.7		29	20.7		36	16.0		
1 lifetime trauma	56	20.4		29	20.7		54	24.0		
2 lifetime traumas	75	27.4		32	22.9		54	24.0		
3 lifetime traumas	42	15.3		35	25.0		46	20.4		
4 or more lifetime traumas	47	17.2		15	10.7		35	15.6		
Resilience (CD-RISC)[2]	274	100	74.2(6.7)	140	100	74.5(5.3)	225	100	74.9(6.3)	.042*
Social Support (MSPSS)[2]	274	100	35.0(4.9)	140	100	35.0(5.3)	225	100	35.0(4.9)	.944
Perceived stress (PSS)[2]	274	100	23.5(5.7)	140	100	21.6(5.9)	225	100	23.5(5.2)	.002**
Rape Stigma (RSS)	274	100	20.5(6.9)	140	100	20.3(6.9)	225	100	21.2(7.0)	.424
Alcohol consumption (AUDIT-C)	274	100	1.8(2.4)	140	100	2.7(2.7)	225	100	2.0(2.4)	.003**
Depression (CES-D)	274	100	32.9(12.8)	140	100	31.8(13.2)	225	100	33.9(12.0)	.293

[1]Modified version [2]Modified response option, *p<.05, **p<.01

Abbreviations: Mean (M); Standard Deviation (SD); Posttraumatic Stress Disorder (PTSD); Davidson Trauma Scale (DTS); Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test – Consumption (AUDIT-C); Center for Epidemiologic Studies Depression Scale (CES-D).

Supplementary Table 2: *Correlation coefficients for baseline pre- and post-assault continuous variables and PTSD scores over time*

	1	2	3	4	5	6	7	8	9	10	11
1. Baseline PTSD (DTS)											
2. 3-month PTSD (DTS)	.44**										
3. 6-month PTSD (DTS)	.28**	.43**									
4. Age	.06	-.04	-.04								
5. Childhood trauma (CTQ-SF)[1]	.19**	.15**	.12**	-.04							
6. Lifetime trauma (LEC)[1]	.14**	.08*	.02	.08*	.27**						
7. Resilience (CD-RISC)[2]	-.02	.03	.08	-.09*	-.06	.01					
8. Social support (MSPSS)[2]	-.05	-.08*	.03	-.06	-.13**	-.08*	.32**				
9. Perceived stress (PSS)[2]	.22**	.12**	.10**	.04	.27**	.12**	-.14**	-.11**			
10. Rape stigma (RSS)	.46**	.32**	.16**	.02	.23**	.11**	-.00	-.11**	.20**		
11. Alcohol use (AUDIT-C)	.09*	.08*	.03	.04	.20**	.24**	-.02	-.01	.09*	.11**	
12. Depression (CES-D)	.62**	.29**	.21**	.05	.16**	.11**	-.05	-.10*	.20**	.35**	.06

* p<.05, **p<.001

[1]Modified version [2]Modified response options

Abbreviations: Posttraumatic Stress Disorder (PTSD); Davidson Trauma Scale (DTS); Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test - Consumption (AUDIT-C); Center for Epidemiologic Studies Depression Scale (CES-D).

Supplementary Table 3: *Childhood and lifetime trauma exposure and PTSD score over time*

			Baseline PTSD score			3-month PTSD score			6-month PTSD score		
	n	%	M(SD)	z	p	M(SD)	z	p	M(SD)	z	p
Childhood neglect				-3.98	.000**		-3.08	.002**		-1.55	.122
No	395	61.8	65.0(25.0)			37.0(23.3)			30.7(20.9)		
Yes	244	38.2	73.0(23.4)			43.1(25.0)			33.3(25.0)		
Childhood domestic violence				-1.75	.080		-2.01	.044*		-1.84	.066
No	554	86.7	67.4(25.1)			38.7(24.2)			31.1(22.1)		
Yes	85	13.3	72.6(21.4)			43.3(23.2)			35.7(25.2)		
Childhood emotional abuse				-3.12	.002**		-3.15	.002**		-3.05	.002**
No	512	80.1	66.6(24.7)			37.9(23.6)			30.7(22.2)		
Yes	127	19.9	73.9(23.9)			44.9(25.3)			35.7(23.8)		
Childhood physical abuse				-2.71	.007**		-1.91	.057		-1.12	.262
No	381	59.6	65.8(25.6)			37.6(23.2)			31.2(22.4)		
Yes	258	40.4	71.4(23.0)			41.7(25.3)			32.3(22.8)		
Childhood sexual abuse				-1.28	.201		-2.61	.009**		-1.73	.084
No	530	82.9	67.4(25.2)			38.2(23.6)			31.2(22.6)		
Yes	109	17.1	71.3(22.0)			44.8(25.8)			34.0(22.5)		
Imprisonment				-0.94	.348		-1.92	.054		-0.20	.841
No	605	94.7	67.8(24.7)			39.0(24.2)			31.7(22.7)		
Yes	34	5.3	72.5(25.2)			45.2(22.4)			30.2(20.8)		
Civil unrest or war				-2.75	.006**		-1.84	.066		-0.96	.337
No	613	95.9	67.6(24.9)			38.9(23.9)			31.5(22.6)		
Yes	26	4.1	80.1(16.3)			49.0(28.2)			34.5(22.5)		
Serious injury				-2.50	.012*		-2.04	.041*		-0.14	.888
No	552	86.4	67.1(24.8)			38.5(23.6)			31.7(22.4)		
Yes	87	13.6	74.1(23.1)			44.6(26.7)			31.4(24.0)		
Being close to death				-2.80	.005**		-0.25	.803		-0.87	.384

No	481	75.3	66.4(25.0)			39.3(24.2)			31.2(22.4)		
Yes	158	24.7	73.2(23.1)			39.4(23.9)			33.0(23.1)		
Murder of family/friend				-2.16	.031*		-1.16	.245		-0.29	.769
No	546	85.4	67.1(24.6)			38.8(23.7)			31.6(22.5)		
Yes	93	14.6	73.5(24.8)			42.3(26.2)			31.8(23.0)		
Unnatural death of family/friend				-0.71	.480		-0.63	.526		-0.78	.434
No	525	82.2	67.6(25.2)			39.0(24.2)			31.4(22.5)		
Yes	114	17.8	70.2(22.4)			40.5(23.8)			32.8(23.2)		
Murder of a stranger				-2.02	.044*		-0.64	.523		-0.70	.482
No	570	89.2	67.3(24.9)			39.0(23.8)			31.6(22.7)		
Yes	69	10.8	74.6(22.5)			41.8(26.8)			32.1(32.2)		
Robbed at gunpoint or knifepoint				-1.20	.230		-1.66	.098		-0.28	.777
No	380	59.5	67.2(25.0)			37.5(22.7)			31.7(22.5)		
Yes	259	40.5	69.3(24.3)			42.0(25.9)			31.6(22.7)		
Kidnapped				-1.49	.135		-0.66	.511		-0.09	.925
No	575	90.0	67.5(24.8)			39.0(23.9)			31.6(22.6)		
Yes	64	10.0	73.5(23.4)			41.9(26.5)			31.8(22.6)		

*p<.05, **p<.01

Abbreviations: Mean (M); Standard Deviation (SD); Posttraumatic Stress Disorder (PTSD)

Supplementary Table 4: *Baseline pre- and post-assault categorical variables and PTSD score over time*

	Baseline PTSD score		3-month PTSD score		6-month PTSD score	
	M(SD)	*p*	M(SD)	*p*	M(SD)	*p*
PTSD (DTS)	68.1(24.7)		39.3(24.1)		31.7(22.6)	
Secondary education completed		.397		.984		.887
No	68.9(25.6)		39.3(25.0)		32.3(24.4)	
Yes	67.4(24.1)		39.3(23.5)		31.2(21.1)	
Relationship status		.131		.706		.513
In a relationship	67.0(24.7)		39.0(23.8)		31.5(22.1)	
Widowed/separated/divorced	73.0		48.7		45.0	
Single	72.3(24.3)		40.4(25.7)		31.7(22.6)	
Employment status		.553		.046*		.071
Employed	66.9(25.6)		35.9(24.6)		29.1(22.2)	
Unemployed	68.4(24.5)		40.2(23.9)		32.4(22.6)	
HIV status		.122		.035*		.788
Positive	69.5(25.0)		40.7(24.2)		32.3(24.7)	
Negative	66.8(24.4)		38.0(24.0)		31.1(20.6)	
Childhood trauma (CTQ-SF)[1]		.000**		.002**		.002**
No childhood trauma	63.4(25.9)		36.3(23.2)		29.7(50.5)	
1 childhood trauma	66.7(25.0)		37.3(23.3)		33.8(24.0)	
2 childhood traumas	72.2(22.8)		41.6(24.4)		29.1(24.1)	
3 or more childhood traumas	75.1(21.6)		45.7(25.6)		35.6(22.6)	
Lifetime traumas (LEC)[1]		.025*		.432		.244
No lifetime traumas	72.7(25.1)		41.3(25.3)		32.7(23.4)	
1 lifetime trauma	63.8(26.8)		37.9(24.2)		33.7(25.2)	
2 lifetime traumas	65.0(25.4)		37.3(22.4)		28.4(21.7)	
3 lifetime traumas	69.1(22.2)		38.9(24.2)		30.5(18.7)	
4 or more lifetime traumas	72.2(21.5)		42.8(25.2)		34.3(23.3)	
Resilience (CD-RISC)[2]		.177		.607		.498
1st quartile	71.7(24.7)		40.0(25.8)		31.3(25.2)	
2nd quartile	65.1(24.5)		37.4(25.1)		31.2(23.0)	
3rd quartile	69.1(24.4)		38.8(22.9)		31.8(19.1)	
4th quartile	67.3(24.8)		40.0(23.1)		32.1(21.8)	
Social Support (MSPSS)[2]		.177		.607		.498
1st quartile	74.3(24.7)		42.2(25.4)		31.7(23.6)	
2nd/3rd quartile	66.1(26.9)		40.6(23.9)		31.7(22.4)	
4th quartile	67.1(23.0)		37.5(23.7)		31.6(22.4)	
Perceived stress (PSS)[2]		.000**		.031*		.002**
1st quartile	63.9(27.9)		38.2(24.3)		30.9(25.6)	
2nd quartile	60.2(25.6)		35.9(24.0)		27.4(20.8)	
3rd quartile	69.4(22.3)		40.0(22.9)		32.5(21.8)	
4th quartile	76.8(20.5)		42.2(25.2)		34.9(21.5)	
Rape Stigma (RSS)		.000**		.000**		.000**
1st quartile	51.9(24.4)		28.5(20.8)		27.7(22.0)	
2nd quartile	61.9(22.5)		36.7(21.4)		32.1(22.1)	
3rd quartile	72.5(20.7)		42.2(23.7)		35.9(23.2)	
4th quartile	82.4(21.1)		47.6(25.9)		31.7(22.6)	

Alcohol use (AUDIT-C)		.036*		.035*		.236
No consumption	65.0(25.2)		36.4(23.8)		30.8(22.8)	
Some consumption	69.8(23.8)		42.7(25.3)		33.6(21.8)	
Hazardous consumption	70.6(24.4)		40.4(24.1)		31.3(22.9)	
Depression (CES-D)		.000**		.000**		.000**
1st quartile	47.5(22.1)		30.0(20.7)		24.9(19.8)	
2nd quartile	61.1(20.2)		38.6(23.4)		31.8(22.2)	
3rd quartile	76.2(18.6)		40.1(22.0)		32.1(21.8)	
4th quartile	88.6(18.2)		48.0(26.5)		37.4(24.5)	

[1]Modified version [2]Modified response options, *p<.05, **p<.01
Abbreviations: Mean (M); Standard Deviation (SD); Posttraumatic Stress Disorder (PTSD); Davidson Trauma Scale (DTS); Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test - Consumption (AUDIT-C); Center for Epidemiologic Studies Depression Scale (CES-D).

Supplementary Table 5: *Baseline pre-assault and post-assault (converted to categorical variables) predictors of PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
Time (Baseline) [1]						
3-months	-28.77	1.87	-15.38	.000**	-32.47	-25.06
6-months	-36.40	1.86	-19.62	.000**	-40.05	-32.73
Age	-0.13	0.18	-0.75	.453	-0.48	0.22
Basic education - not completed[1]						
Completed	0.09	1.67	0.06	.955	-3.19	3.38
Employment - Unemployed[1]						
Employed	-1.30	2.08	-0.63	.531	-539	2.78
Relationship status - in a relationship[1]						
Separated/divorced/widow	-1.06	3.50	-0.30	.761	-7.93	5.80
Single	-1.67	2.10	-0.79	.427	-5.79	2.46
HIV status - negative[1]						
HIV positive	2.21	1.83	1.21	.229	-1.39	5.80
Childhood trauma (CTQ-SF) [2]–none[1]						
1 childhood trauma	-0.20	2.17	-0.09	.925	-4.56	4.05
2 childhood traumas	2.11	2.51	0.84	.401	-2.82	7.03
3 or more childhood traumas	2.34	2.53	0.93	.355	-2.63	7.30
Lifetime trauma (LEC) [2]–none[1]						
1 trauma	-0.01	2.81	-0.00	.998	-5.52	5.51
2 traumas	-3.53	2.62	-1.35	.178	-8.68	1.62
3 traumas	-0.77	2.72	-0.28	.778	-6.12	4.59
4 or more traumas	1.06	2.89	0.37	.715	-4.62	6.73
Resilience (CD-RISC)[3]–1st quartile[1]						
2nd quartile	0.43	2.36	0.18	.857	-4.21	5.06
3rd quartile	2.40	2.77	0.87	.387	-3.04	7.83
4th quartile	0.34	2.16	0.16	.875	-3.90	4.58
Social support (MSPSS)[3] – 1st quartile[1]						
2nd/3rd quartile	-0.31	2.43	-0.13	.898	-5.08	4.46
4th quartile	-0.00	2.28	-0.00	1.000	-4.47	4.47
Perceived stress (PSS)[3] – 1st quartile[1]						
2nd quartile	-2.98	2.41	-1.24	.217	-7.72	1.75
3rd quartile	-0.59	2.38	-0.25	.805	-5.26	4.09
4th quartile	1.01	2.61	0.39	.700	-4.14	6.15
Rape stigma (RSS) – 1st quartile[1]						
2nd quartile	6.57	2.44	2.69	.007**	1.77	11.37
3rd quartile	9.67	2.63	3.67	.000**	4.49	14.84
4th quartile	14.71	2.70	5.44	.000**	9.39	20.03
Alcohol use (AUDIT-C) – none[1]						
Some consumption	0.14	2.21	0.06	.948	-4.21	4.49
Hazardous consumption	0.12	2.05	0.06	.953	-3.72	3.96
Depression (CES-D) – 1st quartile[1]						
2nd quartile	8.37	2.38	3.51	.000**	3.69	13.05

3rd quartile	14.53	2.48	5.85	.000**	9.65	19.42
4th quartile	20.87	2.51	8.32	.000**	15.94	25.79

[1] Reference categories in regression model [2]Modified version [3]Modified response options, **p<.01

Abbreviations: Childhood Trauma Questionnaire Short Form (CTQ-SF); Life Events Checklist (LEC); The Connor-Davidson Resilience Scale (CD-RISC); Multidimensional Scale of Perceived Social Support (MSPSS); Perceived Stress Scale (PSS); Rape Stigma Scale (RSS); Alcohol Use Disorders Identification Test (AUDIT); Center for Epidemiologic Studies Depression Scale (CES-D).

Supplementary Table 6: *Interaction between depression quartiles, rape stigma quartiles and least squares mean PTSD score over time*

	PTSD Mean	Std error	*z*	*p*	95% CI Lower	95% CI Upper
Baseline x 1st quartile depression (CES-D)	50.50	1.97	25.61	.000	46.63	54.36
Baseline x 2nd quartile depression	62.41	1.89	32.98	.000	58.70	66.11
Baseline x 3rd quartile depression	77.74	1.96	39.76	.000	73.91	81.29
Baseline x 4th quartile depression	83.51	1.93	43.29	.000	79.73	87.29
3-months x 1st quartile depression	33.51	2.07	16.19	.000	29.46	37.57
3-months x 2nd quartile depression	39.04	1.99	19.64	.000	35.14	42.93
3-months x 3rd quartile depression	40.36	2.05	19.65	.000	36.34	44.39
3-months x 4th quartile depression	45.52	2.02	22.51	.000	41.56	49.49
6-months x 1st quartile depression	27.57	2.34	11.78	.000	22.98	32.16
6-months x 2nd quartile depression	32.74	2.25	14.55	.000	28.33	37.15
6-months x 3rd quartile depression	30.59	2.32	13.16	.000	26.03	35.14
6-months x 4th quartile depression	36.09	2.28	15.82	.000	31.62	40.56
1st quartile stigma (RSS) x						
Baseline x 1st quartile depression	42.11	2.28	18.48	.000	37.64	46.57
Baseline x 2nd quartile depression	54.01	2.27	23.75	.000	49.55	58.47
Baseline x 3rd quartile depression	69.35	2.39	29.05	.000	64.67	74.94
Baseline x 4th quartile depression	75.12	2.46	30.51	.000	70.29	74.02
3-months x 1st quartile depression	25.12	2.4	10.63	.000	20.49	29.76
3-months x 2nd quartile depression	30.64	2.4	13.01	.000	26.03	35.26
3-months x 3rd quartile depression	31.97	2.5	12.95	.000	27.13	36.81
3-months x 4th quartile depression	37.13	2.5	14.64	.000	32.16	42.10
6-months x 1st quartile depression	19.17	2.6	7.36	.000	14.07	24.28
6-months x 2nd quartile depression	24.35	2.6	9.44	.000	19.29	29.40
6-months x 3rd quartile depression	22.19	2.7	8.23	.000	16.90	27.48
6-months x 4th quartile depression	27.70	2.7	10.08	.000	22.32	33.08
2nd quartile stigma x						
Baseline x 1st quartile depression	49.05	2.22	22.05	.000	44.69	53.41
Baseline x 2nd quartile depression	60.95	2.19	27.78	.000	56.65	65.25
Baseline x 3rd quartile depression	76.29	2.32	32.92	.000	71.74	80.83
Baseline x 4th quartile depression	82.06	2.34	35.14	.000	77.48	86.83
3-months x 1st quartile depression	32.06	2.31	13.87	.000	27.53	36.59
3-months x 2nd quartile depression	37.59	2.28	16.51	.000	33.12	42.05
3-months x 3rd quartile depression	38.91	2.40	16.21	.000	34.20	43.61
3-months x 4th quartile depression	44.07	2.41	18.26	.000	39.34	48.80
6-months x 1st quartile depression	26.12	2.56	10.22	.000	21.11	31.13
6-months x 2nd quartile depression	31.29	2.51	12.47	.000	26.37	36.21
6-months x 3rd quartile depression	29.13	2.64	11.05	.000	23.97	34.30
6-months x 4th quartile depression	34.64	2.63	13.15	.000	29.48	39.80
3rd quartile stigma x						
Baseline x 1st quartile depression	53.30	2.33	22.90	.000	48.74	57.87
Baseline x 2nd quartile depression	65.21	2.23	29.22	.000	60.84	69.59
Baseline x 3rd quartile depression	80.54	2.22	36.33	.000	76.20	84.89
Baseline x 4th quartile depression	86.32	2.18	39.52	.000	82.04	90.60
3-months x 1st quartile depression	36.32	2.41	15.06	.000	31.59	41.05
3-months x 2nd quartile depression	41.84	2.31	18.09	.000	37.31	46.38
3-months x 3rd quartile depression	43.17	2.30	18.73	.000	38.65	47.68
3-months x 4th quartile depression	48.33	2.27	21.31	.000	43.88	52.77
6-months x 1st quartile depression	30.37	2.65	11.48	.000	25.19	35.56
6-months x 2nd quartile depression	35.55	2.54	13.98	.000	30.56	40.53
6-months x 3rd quartile depression	33.39	2.55	13.10	.000	28.40	38.39
6-months x 4th quartile depression	38.90	2.50	15.55	.000	34.00	43.80

4th quartile stigma x						
Baseline x 1st quartile depression	57.54	2.41	23.86	.000	52.81	62.27
Baseline x 2nd quartile depression	69.45	2.27	30.54	.000	64.99	73.90
Baseline x 3rd quartile depression	84.78	2.26	37.44	.000	80.34	89.22
Baseline x 4th quartile depression	90.55	2.10	43.10	.000	86.43	94.67
3-months x 1st quartile depression	40.55	2.49	16.27	.000	35.67	45.44
3-months x 2nd quartile depression	46.08	2.35	19.58	.000	41.46	50.69
3-months x 3rd quartile depression	47.10	2.35	20.17	.000	42.79	52.01
3-months x 4th quartile depression	52.56	2.19	24.03	.000	48.28	56.85
6-months x 1st quartile depression	34.61	2.72	12.72	.000	29.27	39.94
6-months x 2nd quartile depression	39.78	2.58	15.42	.000	34.72	44.84
6-months x 3rd quartile depression	37.62	2.59	14.53	.000	32.55	42.70
6-months x 4th quartile depression	43.13	2.43	17.76	.000	38.37	47.89

Abbreviations: Center for Epidemiologic Studies Depression Scale (CES-D); Rape Stigma Scale (RSS).

CHAPTER 4: SUPPLEMENTARY MATERIAL

METHODS

Parent study

A subset of participants (n=96), recruited from a longitudinal cohort study investigating the impact of rape on women's health and their use of health services in South Africa (n = 852), was used in this study. The study is also known as the Rape Impact Cohort Evaluation (RICE) study [1]. Female survivors of rape were recruited from rape centres in and around the city of Durban located in the KwaZulu Natal province of South Africa. The rape centres provide comprehensive emergency care, including access to police, counselling, and medical and forensic care.

Clinical measures

The Mini International Neuropsychiatric Interview

The Mini International Neuropsychiatric Interview (MINI) is a structured psychiatric interview that screens for 16 DSM-IV psychiatric disorders including mood disorders, anxiety disorders, alcohol and drug dependence, psychosis, eating disorders and personality disorders [2]. The MINI was used at baseline to screen for PTSD based on prior traumas other than the rape. The MINI has shown good reliability and validity in various settings [3,4].

Davidson Trauma Scale

The Davidson Trauma Scale (DTS) is a self-report questionnaire used to assess seventeen PTSD symptoms [5]. The DTS was administered at all timepoints and responses were measured on a 5-point Likert scale for symptom frequency (ranging from 0 'not at all' to 4 'every day') and symptom severity (ranging from 0 'not at all distressing' to 4 'extremely distressing'). The symptom frequency and severity scores were added together to produce a PTSD total score ranging between 0 and 136. A total score of forty or more is considered indicative of PTSD. Previous findings indicated that the DTS was excellent at discriminating between participants with and without PTSD at a cut-point of 40 [5,6]. The DTS showed excellent reliability in this study at each timepoint with a Cronbach alpha sore of .92 at baseline, .91 at 3-months and .93 at 6-months post-rape.

Childhood Trauma Questionnaire Short Form

A modified version of the Childhood Trauma Questionnaire Short Form (CTQ-SF) was used to measure exposure to childhood trauma before the age of eighteen years [7,8]. The fourteen items measuring childhood trauma centres around sexual abuse, physical abuse, emotional abuse, parental neglect and domestic violence. Responses were measured on a 4-point Likert scale ranging from 1 'never' to 4 'very often'. The CTQ-SF has shown excellent validity in previous studies [9,10] and showed acceptable reliability in this study with a Cronbach alpha score of .75 at baseline.

Life Events Checklist

A modified version of the Life Events Checklist (LEC) was used to measure lifetime exposure to different trauma types at the baseline visit [11,12]. The modified version of the LEC measures direct exposure to nine trauma types using a dichotomous 'yes/no' response. The trauma types measured were imprisonment, civil unrest/war, serious injury, being close to death, murder of a family member or friend, unnatural death of a family member or friend, murder of a stranger/s, robbed at gunpoint or knifepoint and kidnapping. The number of yes responses are added together to yield a total score ranging from 0 to 9 and indicating the trauma load or number of traumas exposed to during the participant's lifetime.

Alcohol Use Disorders Identification Test (AUDIT)

The original Alcohol Use Disorders Identification Test (AUDIT) is a 10-item, self-report questionnaire and responses are recorded on a five-point Likert scale using different response options relevant to the different items [13]. The AUDIT-C is a sub-scale of the original AUDIT and is used to measure alcohol consumption [14]. A score of 3 or more on the AUDIT-C indicates hazardous drinking in women. The AUDIT was developed by the World Health Organisation (WHO) and has shown good reliability and validity in various settings and cultures [15–21]. The AUDIT-C showed good reliability in this study at each timepoint with a Cronbach alpha sore of .83 at baseline, .86 at 3-months post-rape and .83 at 6-months post-rape.

Center for Epidemiologic Studies Depression Scale (CES-D)

The Center for Epidemiologic Studies Depression Scale (CESD) is a 20-item, self-report questionnaire used to screen for current depression in accordance with DSM-IV criteria [22]. The CESD was completed at all time points. Responses were recorded on a 4-point Likert scale

ranging from 0 ‘rarely or none of the time’ to 3 ‘most or all of the time’. The CESD has shown good reliability and validity in various cross-cultural samples and in clinic and community settings [23–32]. The CES-D showed good reliability in this study at each timepoint with a Cronbach alpha sore of .89 at baseline, .88 at 3-months and .89 at 6-months post-rape.

Procedure

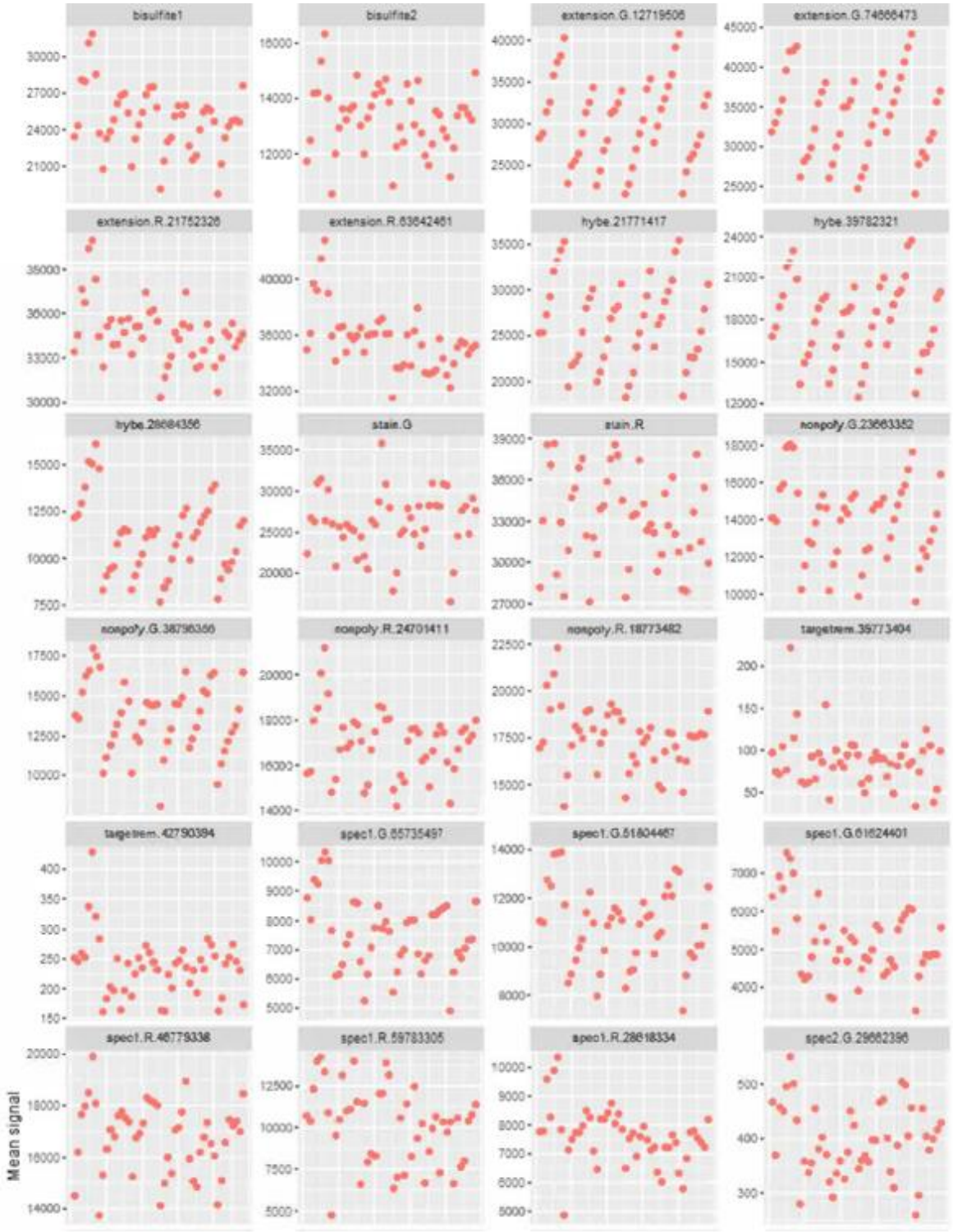
Epigenome-wide association study

The Illumina MethylationEPIC BeadChip array was used to investigated genome-wide differences in methylation between participants with and without PTSD (Illumina, California, United States). The array interrogates over 850 000 sites at single-nucleotide resolution covering 99% of RefSeq genes and including CpG islands, shores and shelves. Results obtained from the EPIC are highly reproducible (98%) with a less than 1% false positive rate reported Illumina, 2012, 2015a).

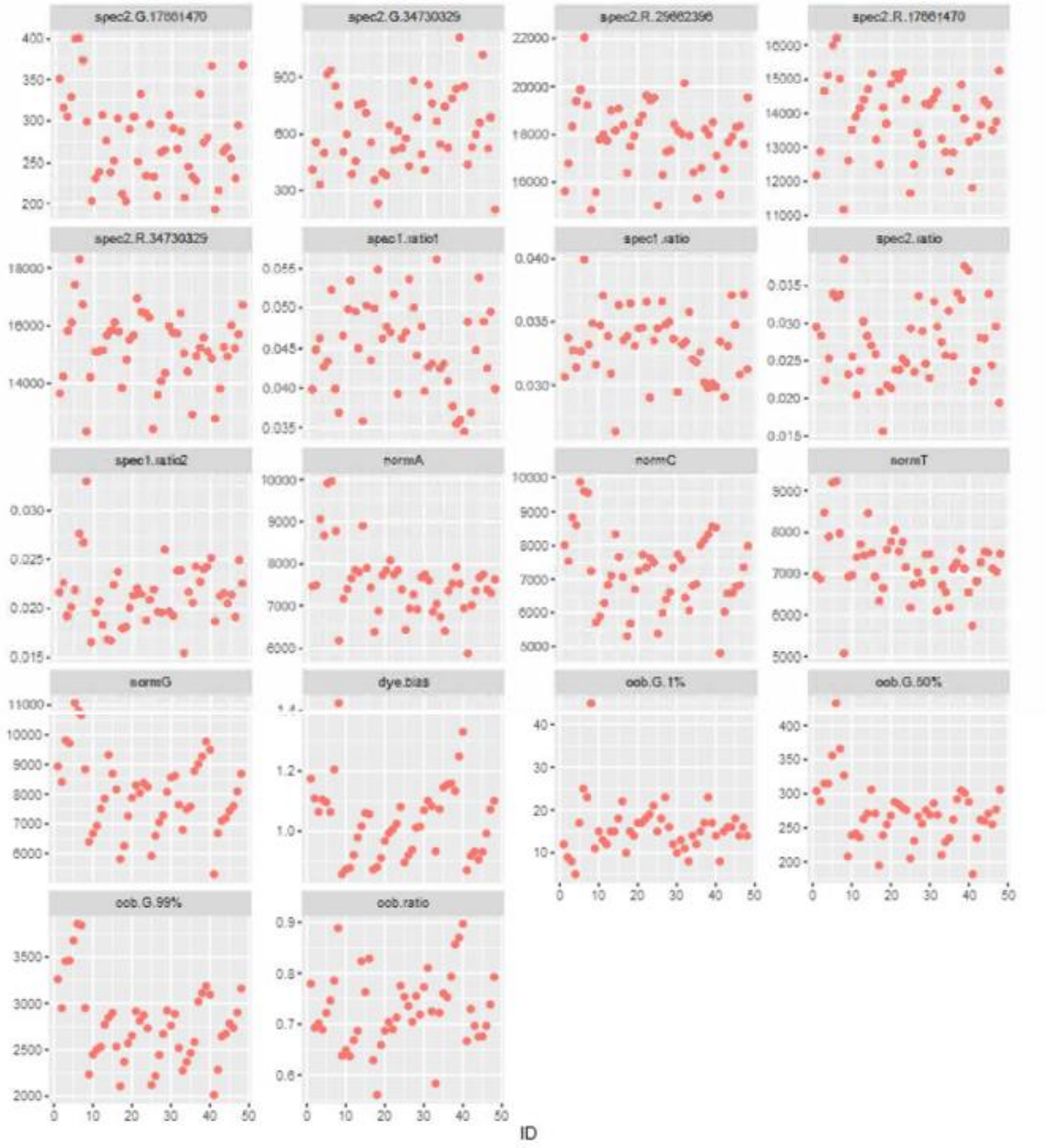
DNA was extracted from peripheral blood samples using the Gentra Puregene DNA extraction kit (Qiagen, Germany) and quantified by fluorimetry, using the PicoGreen dsDNA quantitation reagent (ThermoFisher Scientific, Massachusetts, United States). Sample concentrations were normalised to 50 ng/µl and shipped on dry ice to the Epigenome Centre at the University of South California (USC) in Los Angeles, United States. The DNA samples were bisulfite-converted using the Zymo EZ DNA Methylation Kit (Zymo Research, California, United States) and assayed using the Illumina MethylationEPIC BeadChip (Illumina, California, United States). The samples were randomly assigned to one of six arrays (8 samples per array) to reduce technical bias.

Internal control probes

There are 42 internal control probes present on the MethylationEPIC array which were used to assess the quality of the sample processing steps e.g. bisulfite conversion, hybridization, staining, extension, specificity and target removal [34]. There were no outliers detected i.e. none of the samples had a mean score > 5 standard deviations from the expected mean on all control probes (see Supplementary Figure 1). This indicates that all steps of the sample processing procedures were successful for all samples and that the arrays used in this study passed the sample processing quality control measures.



Supplementary Figure 1: Distribution of probe intensity signals for the MethylationEPIC control probes. The *x*-axis represents samples listed from 1 to 48 and the *y*-axis represents the mean signal intensities on each control probe.

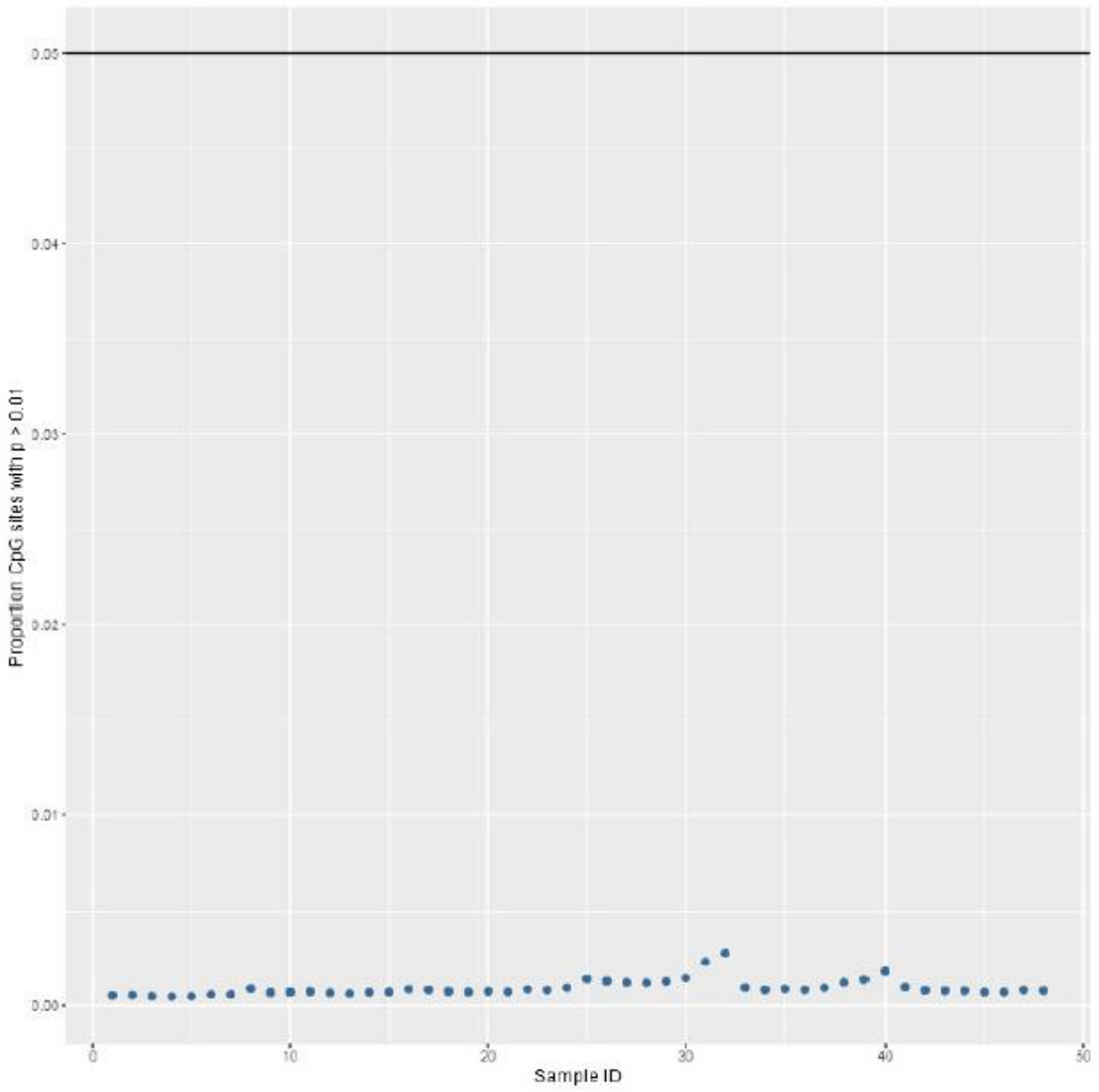


Supplementary Figure 1 (continued): Distribution of probe intensity signals for the MethylationEPIC control probes. The *x*-axis represents samples listed from 1 to 48 and the *y*-axis represents the mean signal intensities on each control probe.

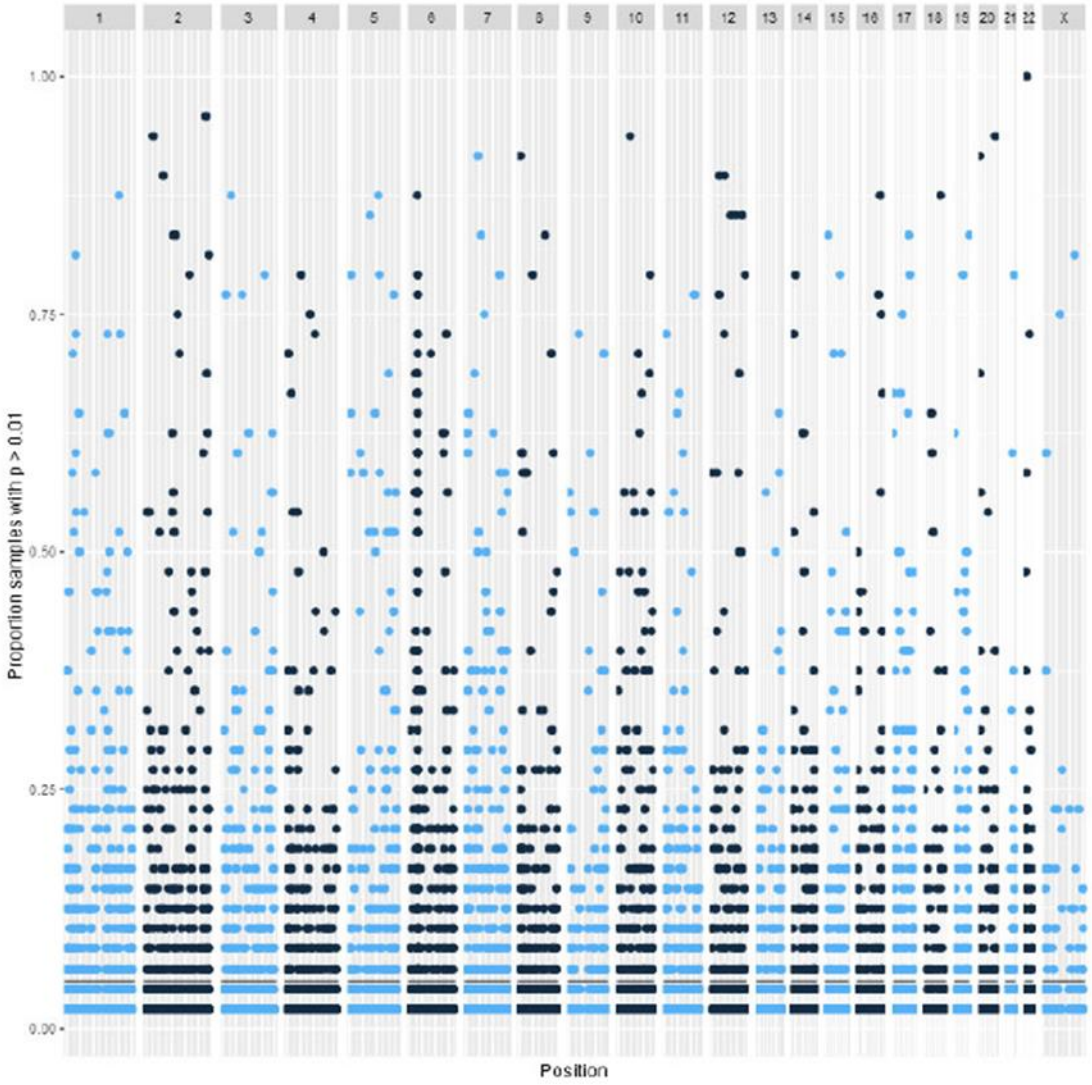
Negative control probes

The array also contains 600 negative control probes i.e. randomly permutated sequences that should not hybridise to sample DNA [35]. However, cross-hybridisation may occur as a result of the simplification of the DNA code after bisulfite conversion or due to DNA polymorphisms/mutations [36]. Nonetheless, the mean fluorescence intensity signal across negative control probes is considered to be background noise [37]. A detection p-value is calculated for every CpG site on the MethylationEPIC array and in every sample using a signal-to-noise ratio of fluorescence intensities where signal represents target probe intensities and noise represents negative probe intensities [35]. A small detection *p*-value ($p < 0.01$) indicates that methylated/unmethylated intensities are likely true intensities and not as a result of background noise [34]. The null hypothesis is that the sum of methylated and unmethylated intensities is equal to background intensities. If $p < 0.01$ the null hypothesis can be rejected. The detection *p*-value is used to evaluate both the quality of the samples and the technical quality of the performance of the array [35]. The proportion of samples showing a detection *p*-value > 0.01 was less than 5% indicating that all samples passed the quality check (see Supplementary Figure 3). There were 3521 probes showing a detection *p*-value > 0.01 in more than 5% of the samples indicating that the data resulting from these probes were unreliable (see Supplementary Figure 2).

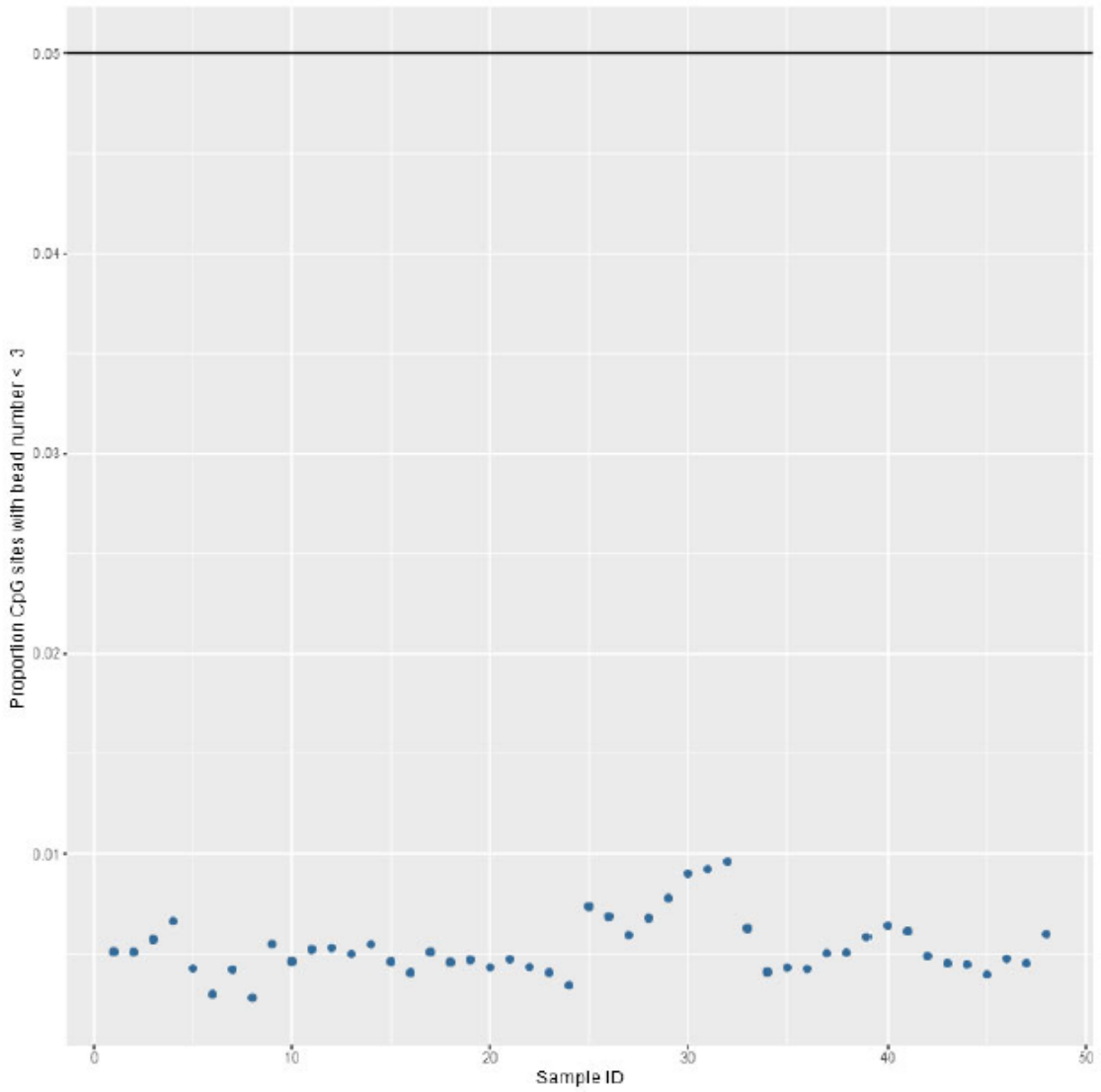
The array also contains multiple beads containing hundreds to thousands of oligonucleotides designed to target the same CpG site in sample DNA [35]. Basing findings on the average intensities across multiple beads increases the reliability of the findings since the procedure is essentially repeated several times [34]. The beads are randomly distributed across the array to minimise batch effects associated with the position of a bead on the array. A 'number of beads threshold' of < 3 (i.e. number of beads detecting methylated/unmethylated signal intensities) is used to evaluate both the quality of the samples and the technical quality of the performance of the array [35]. Less than 5% of the samples showed a high proportion of probes with a number of beads threshold < 3 indicating that all samples passed this quality check (see Supplementary Figure 4). There were 26415 probes with a number of beads threshold < 3 in more than 5% of the samples (see Supplementary Figure 5). The 3521 CpG probes showing a detection *p*-value > 0.01 and the 26415 CpG probes with a number of bead threshold < 3 were excluded from the downstream analysis.



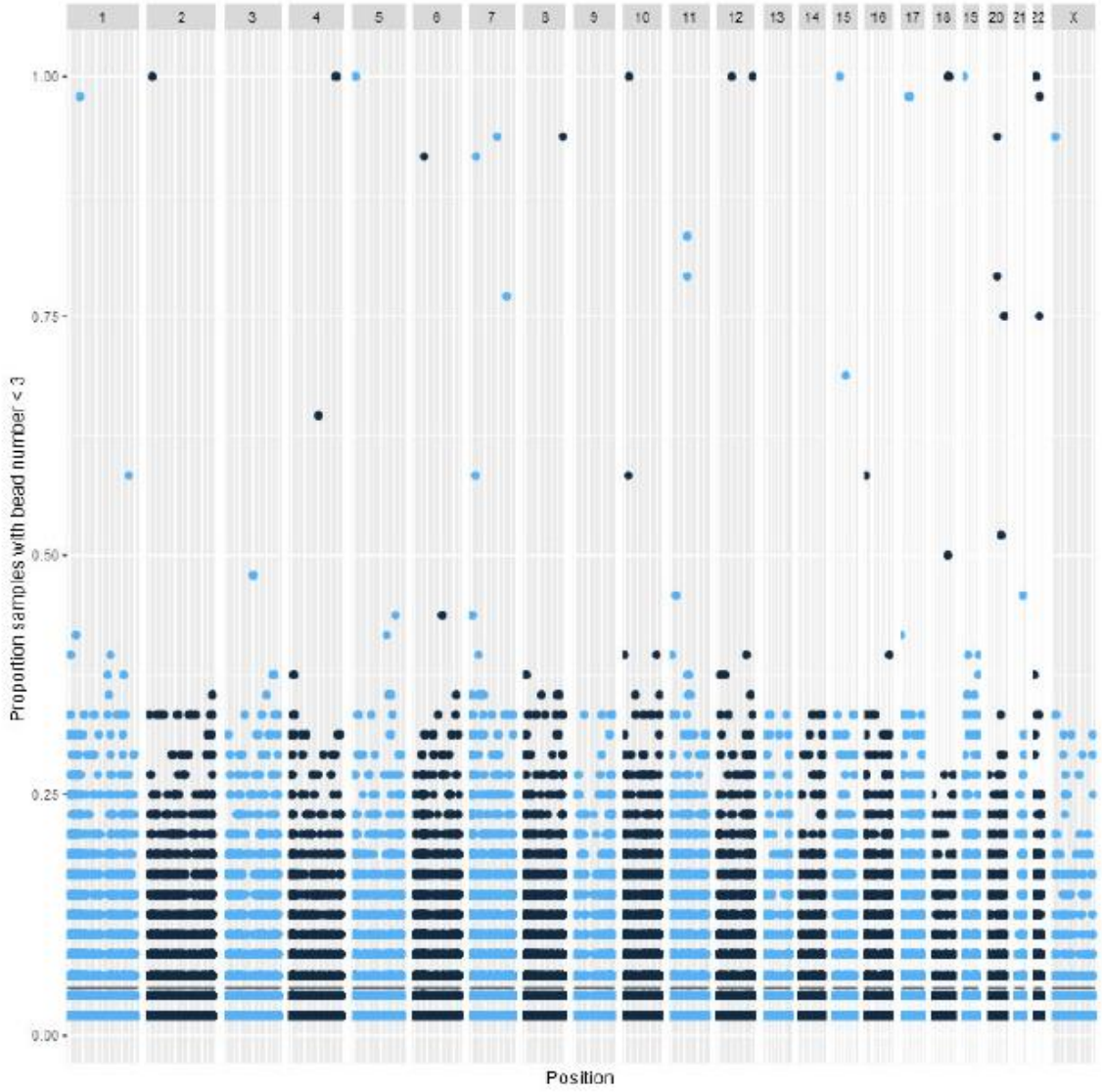
Supplementary Figure 2: All samples showed a detection *p*-value > 0.01 indicating that the methylated/unmethylated intensities are likely true intensities and not the result of background noise. The *x*-axis represents samples ordered from 1 to 48 and the *y*-axis represents the detection *p*-value.



Supplementary Figure 3: Distribution of the probes indicating that 3521 probes showed a detection *p*-value > 0.01 in more than 5% of the samples. The *x*-axis represents the chromosomal position of the probe and the *y*-axis represents the detection *p*-value.



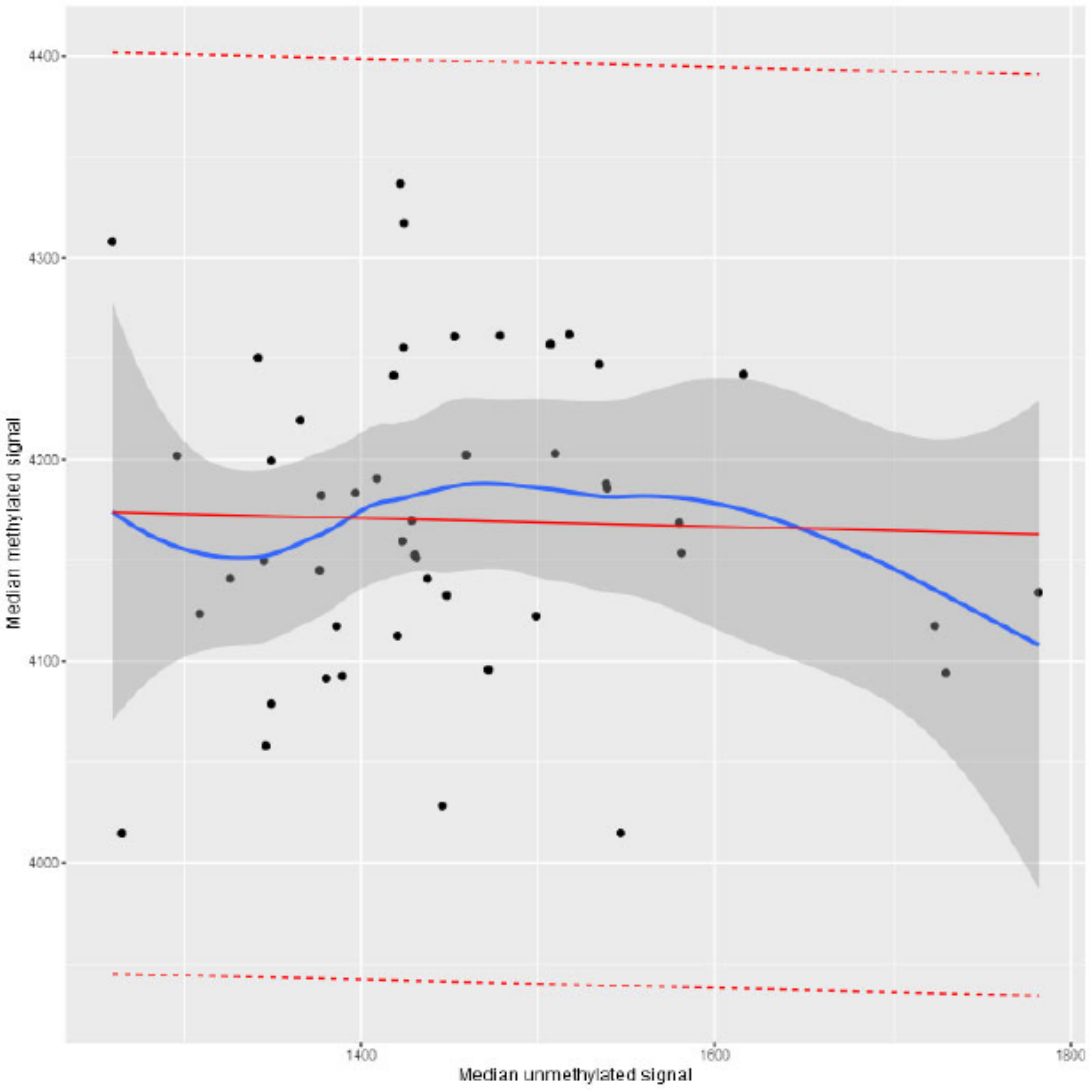
Supplementary Figure 4: Less than 5% of the samples showed a high proportion of probes with a number of beads threshold < 3 indicating that all samples passed this quality check. The *x*-axis represents samples ordered from 1 to 48 and the *y*-axis represents the proportion of probes for each sample for which less than 3 beads were detected.



Supplementary Figure 5: There were 26415 probes with a number of beads threshold < 3 in more than 5% of the samples. The *x*-axis represents the chromosomal position of the probes and the *y*-axis represents the proportion samples for which a given probe failed to be detected in at least 3 beads.

Median intensities

To further assess the quality of sample DNA, median methylated intensities were compared to the median unmethylated intensities across the array and a sample was considered an outlier if the median methylated/unmethylated intensities were more than 3 standard deviations from the expected median (see Supplementary Figure 6). A sample may show increased methylated/unmethylated signals if it is contaminated or if the DNA concentration was too low for sufficient binding to probes therefore indicating poor quality of the sample DNA [38]. There were no outliers for the methylated vs unmethylated intensity comparison. No samples were excluded from the analysis given that all samples passed the quality control checks.



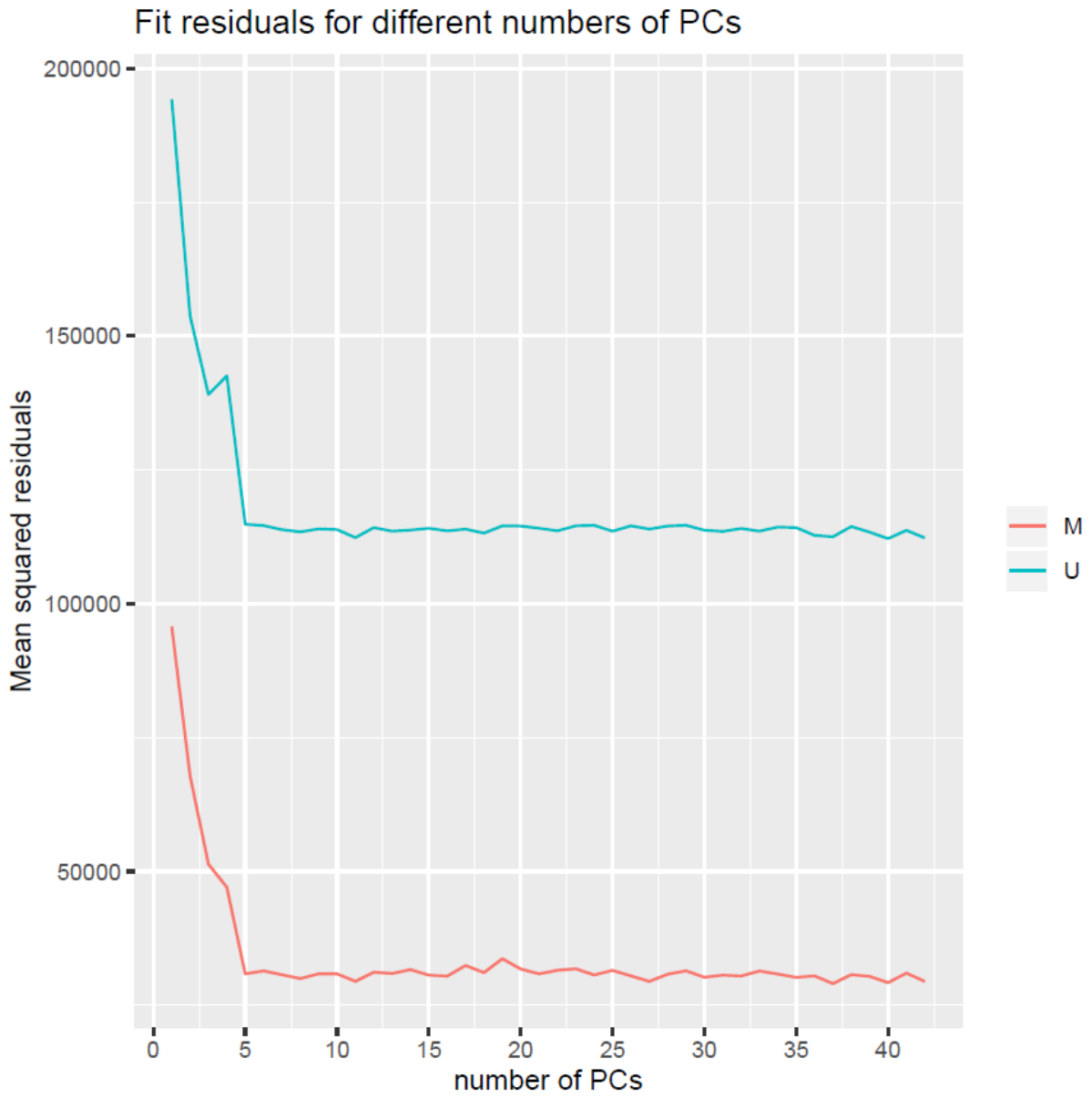
Supplementary Figure 6: Distribution of median methylation intensities for each sample. The solid red line represents the regression line and the solid blue line represents the loess-smoothed line resulting from the observed intensities. The upper and lower dotted red lines enclose the points at most 3 standard deviations from the expected mean. The shaded region denotes the 95% confidence interval around the loess-smoothed line. The *x*-axis represents the median unmethylated intensities and the *y*-axis represents the median methylated intensities.

Functional normalisation and batch effects

The array includes two different assay types, the first being the Infinium I assay which contains two probes (one for methylated intensities and one for unmethylated intensities) to target a CpG site. The second assay type, the Infinium II, contains only one probe to distinguish between methylated and unmethylated alleles using green and red dye colours [35]. The different technological designs used in Infinium I and II assays could potentially introduce technical bias since an upwards shift in β-values for unmethylated intensities and a downwards shift for methylated intensities have been associated with the Infinium II assay when comparing the two assays with each other [34].

Differences in methylation intensities have been associated with batch effects in previous studies e.g. the assignment of a sample to a given plate or array and positioning the sample in a given row or column [39]. Column assignment was not considered as a potential batch effect in this study since the MethylationEPIC array does not contain columns like its predecessors. All samples in this study were located on the same plate. Six arrays were used to accommodate the 48 samples analysed in this study (8 samples per array). Each sample was assigned to one of eight rows on each array.

Functional normalisation was used in an effort to reduce the technical bias associated with batch effects by using the information obtained from the 42 control probes on the MethylationEPIC array [37]. This method removes the variance associated with technical variation without compromising the variance associated with methylation differences between PTSD cases and controls since none of the control probes are designed to detect a biological signal [37]. Five principal components (PCs) were found to explain most of the technical variance in the sample (see Supplementary Figure 7) and were regressed against the batch variables array and row position before and after functional normalisation [40].



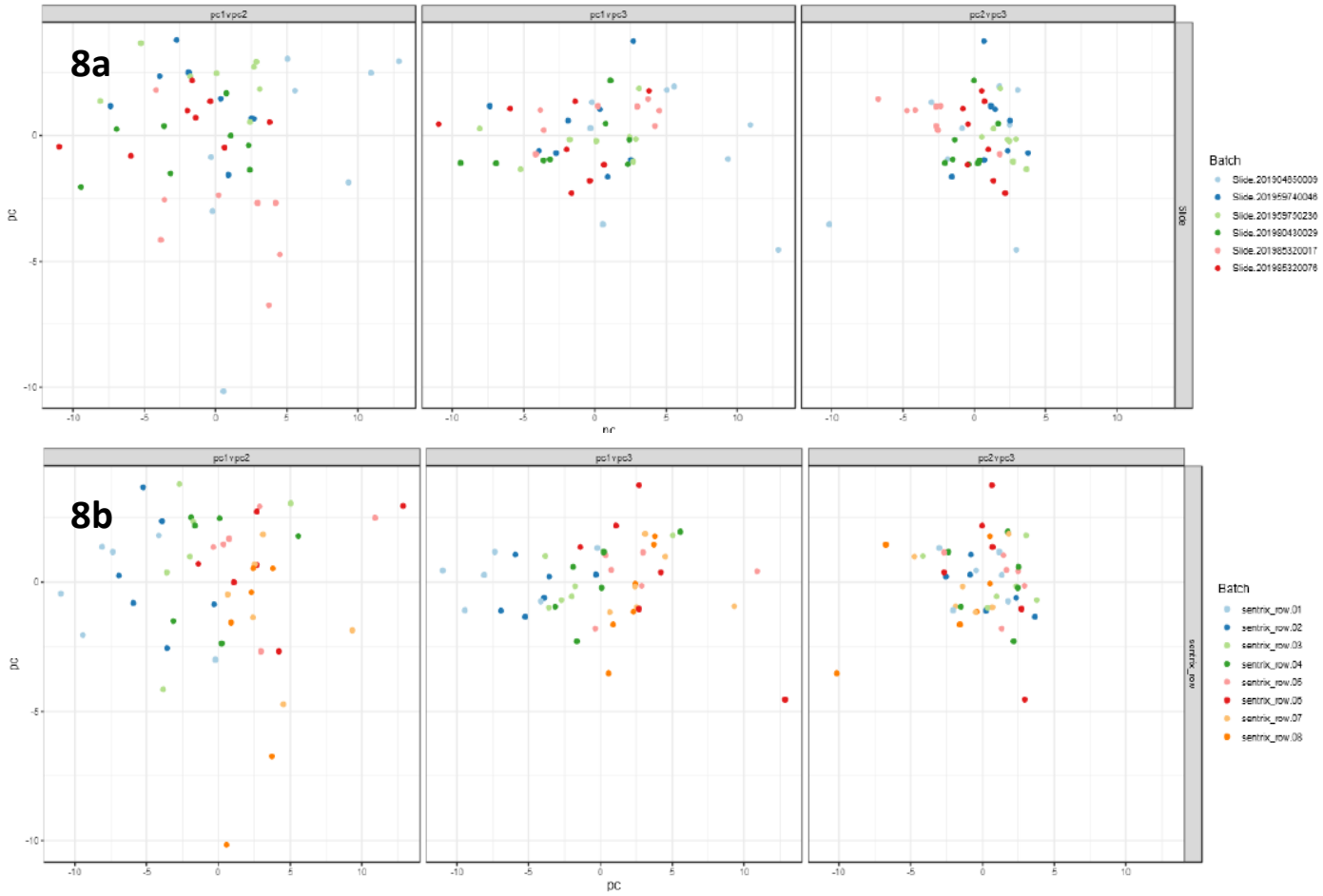
Supplementary Figure 7: Estimating the number of principal components (PCs) to regress against batch variables. The first five PCs explained most of the technical variance observed from the methylated (M) and unmethylated (U) intensities. Variance explained was estimated using cross-validation. The *x*-axis represents the number of PCs and the *y*-axis represents the mean squared residuals/variance.

Batch effects before functional normalisation

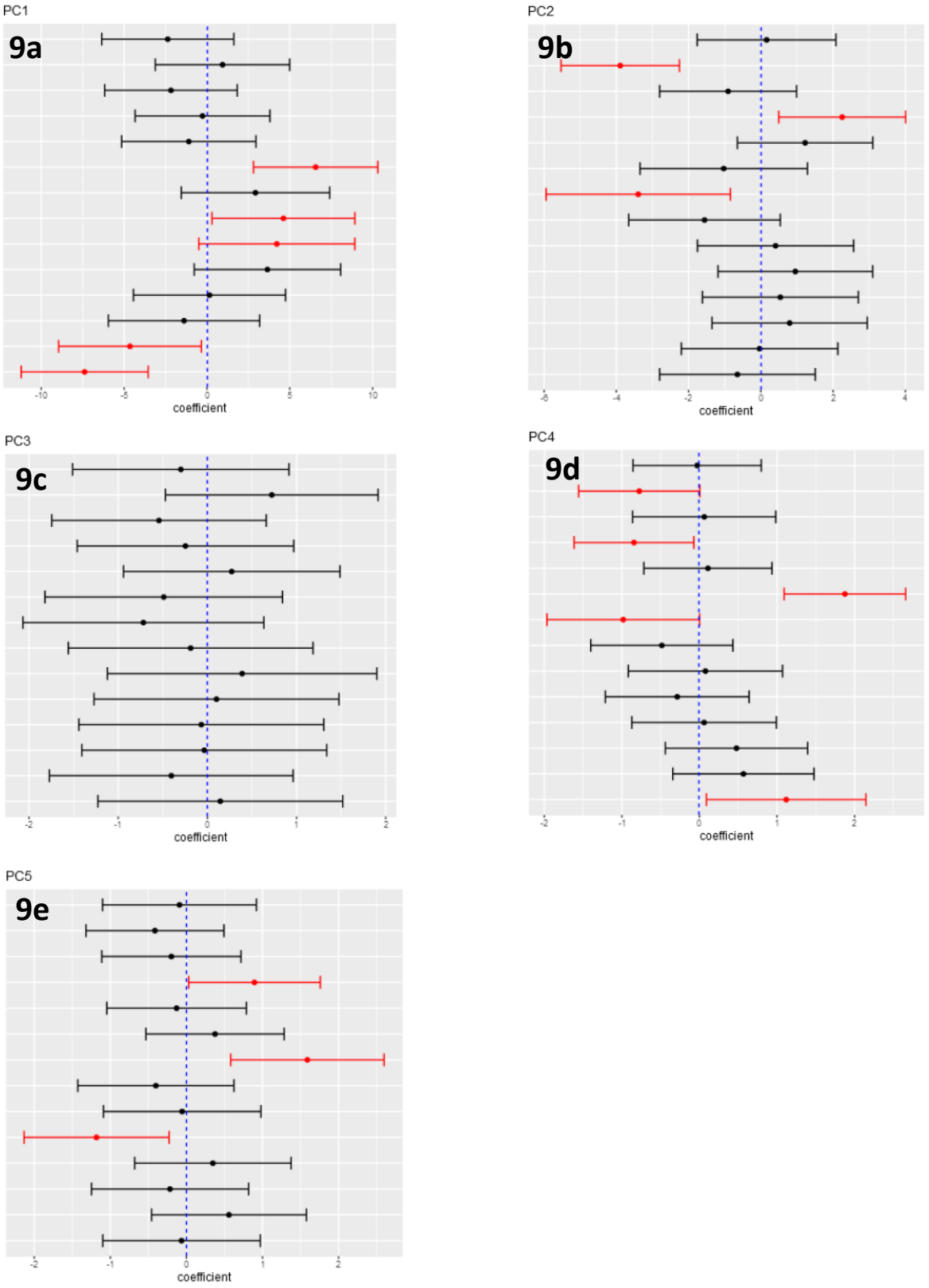
Each control probe PC was regressed against each level of each batch variable (see Supplementary Figures 8a-8b and Supplementary Figures 9a-9e) to determine the extent to which control variation accounted for batch variation. There were six slide batch variables representing the six arrays used in the epigenome-wide association study (EWAS; slide 201904850009, slide 201959740048, slide 201959750230, slide 201990430029, slide 201985320017, slide 201995320078) and eight row batch variables representing the sentrix row on an array to which a sample was assigned (sentrix row 01 – sentrix row 08). The significant findings resulting from ANOVAS and post-hoc t-tests comparing PC values between batch variables are presented in Supplementary Table 1.

Supplementary Table 1: *Association between principal components and batch variables before functional normalisation*

Batch variable	Batch variable subcategory	Principal Component (PC)	*F*	*t*	*p*	95% Confidence Interval (CI) Lower	Upper
Slide		PC1	3.54		.009		
	210904650009	PC1		6.56	.0002	2.81	10.32
Row		PC1	7.91		.000005		
	Sentrix row 01	PC1		-7.38	.00006	-11.21	-3.55
	Sentrix row 02	PC1		-4.64	.017	-8.95	-0.33
	Sentrix row 06	PC1		4.22	.045	-0.49	8.93
	Sentrix row 07	PC1		4.62	.018	0.31	8.93
Slide		PC2	5.06		.001		
	201959750236	PC2		2.25	.005	0.50	4.01
	201985320017	PC2		-3.89	.000002	-5.53	-2.26
Row	Sentrix row 08	PC2		-3.39	.004	-5.95	-0.84
Slide		PC4	6.35		.0002		
	210904650009	PC4		1.87	.000002	1.09	2.66
	201985320017	PC4		-0.78	.028	-1.56	0.01
Row	Sentrix row 01	PC4		1.12	.016	0.01	2.15
	Sentrix row 08	PC4		-0.98	.027	-1.97	0.00
Slide	201959750236	PC5		0.90	.022	0.03	1.76
Row		PC5	3.31		.007		
	Sentrix row 05	PC5		-1.18	.007	-2.14	-0.23
	Sentrix row 08	PC5		1.59	.0007	0.58	2.60



Supplementary Figures 8a & 8b: Principal component (PC) plots for the six slides used in the EWAS and the eight rows to which samples were assigned. The figure is divided into three parts representing the first three PCs. Each dot represents one of the 48 samples included in the EWAS and each colour represents one of the six arrays used in the EWAS in Supplementary Figure 9a and each of the eight rows in Supplementary Figure 9b.



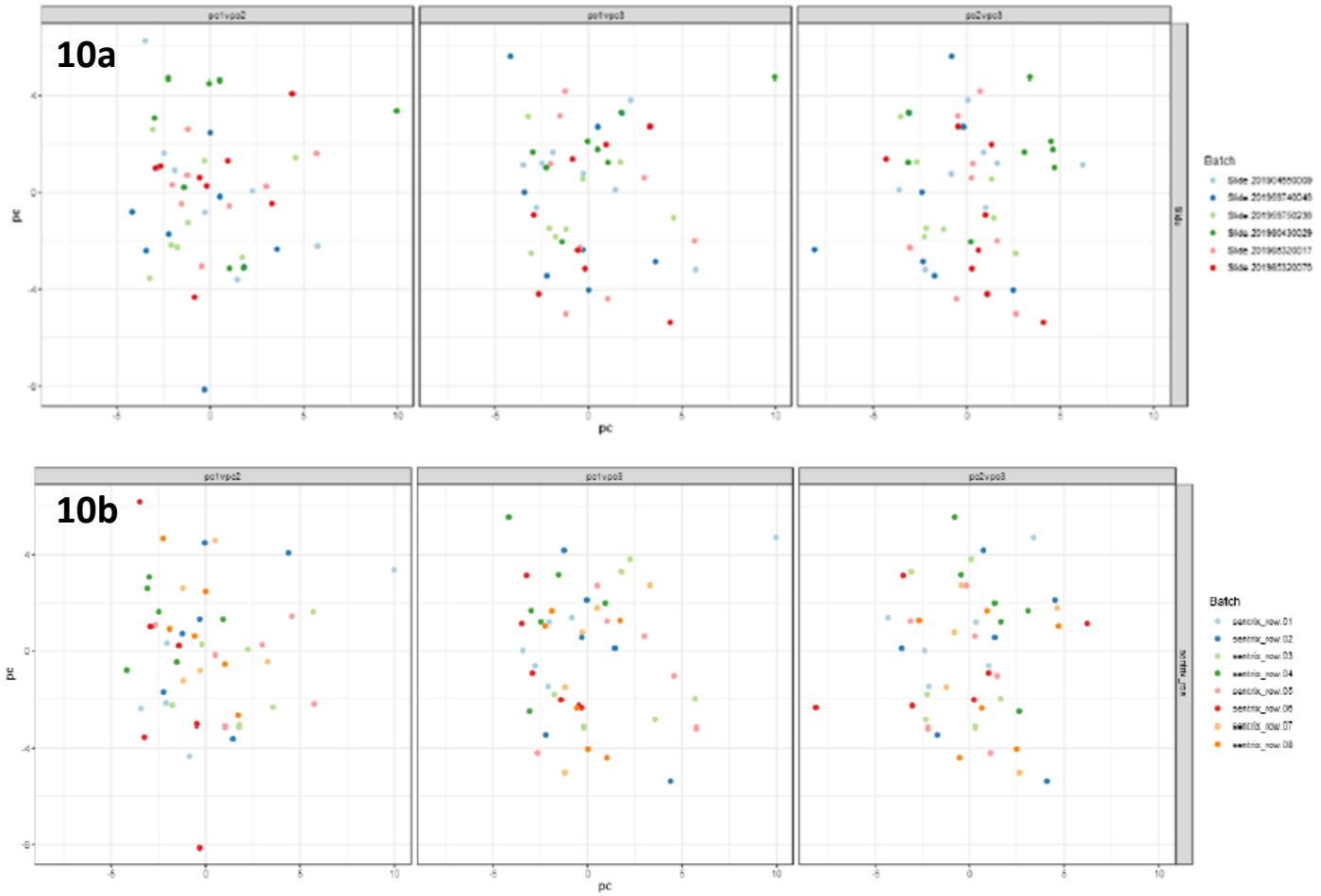
Supplementary Figures 9a-e: Forrest plots representing regression coefficients and 95% confidence intervals (*x*-axis) before functional normalisation for each slide (bar 1 to 6) and each row (bar 7 to 14) divided into five figures representing principal component 1 (PC1) (Supplementary Figure 9a), PC2 (Supplementary Figure 9b), PC3 (Supplementary Figure 9c), PC4 (Supplementary Figure 9d) and PC5 (Supplementary Figure 9e).

Batch effects after functional normalisation

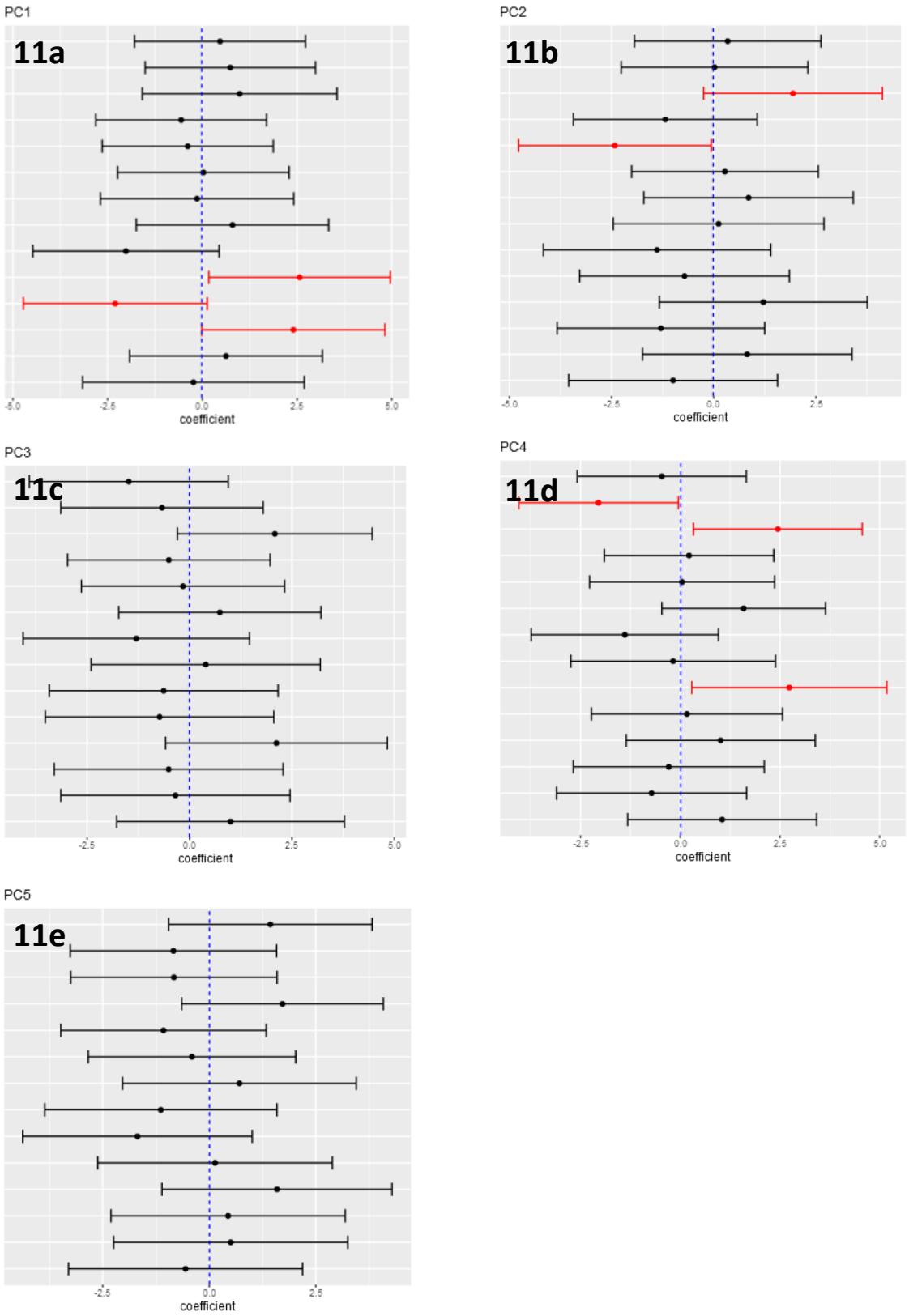
Principal components analysis was then applied to the normalized DNA methylation matrix. Each top PC was regressed against each level of each batch variable following functional normalisation (see Supplementary Figures 10a-10b and Supplementary Figures 11a-11e) to evaluate the extent of technical variation remaining in the normalized data. None of the ANOVAS comparing PC values with slide and row batch variables were statistically significant, but post-hoc t-test comparisons did reveal some significant findings between PC values and slide/row numbers. The results of the significant t-tests are presented in Supplementary Table 2.

Supplementary Table 2: *Association between principal components and batch variables after functional normalisation*

Batch variable	Batch variable subcategory	Principal Component (PC)	*t*	*p*	95% Confidence Interval (CI) Lower	Upper
Row	Sentrix row 03	PC1	2.40	.027	-0.01	4.82
	Sentric row 04	PC1	-2.30	.035	-4.73	0.13
	Sentrix row 05	PC1	2.57	.018	0.17	4.96
Slide	201959740046	PC2	-2.42	.023	-4.78	-0.06
	201980430029	PC2	1.94	.048	-0.25	4.12
	201980430029	PC4	2.45	.011	0.33	4.56
	201985320017	PC4	-2.05	.023	-4.05	.0.05
Row	Sentrix row 06	PC4	2.73	.014	0.29	5.18



Supplementary Figures 10a & 10b: Principal component (PC) plots following functional normalisation for the six slides used in the EWAS and the eight rows to which samples were assigned. The figure is divided into three parts representing the first three PCs. Each dot represents one of the 48 samples included in the EWAS and each colour represents one of the six arrays used in the EWAS in Supplementary Figure 10a and each of the eight rows in Supplementary Figure 10b.



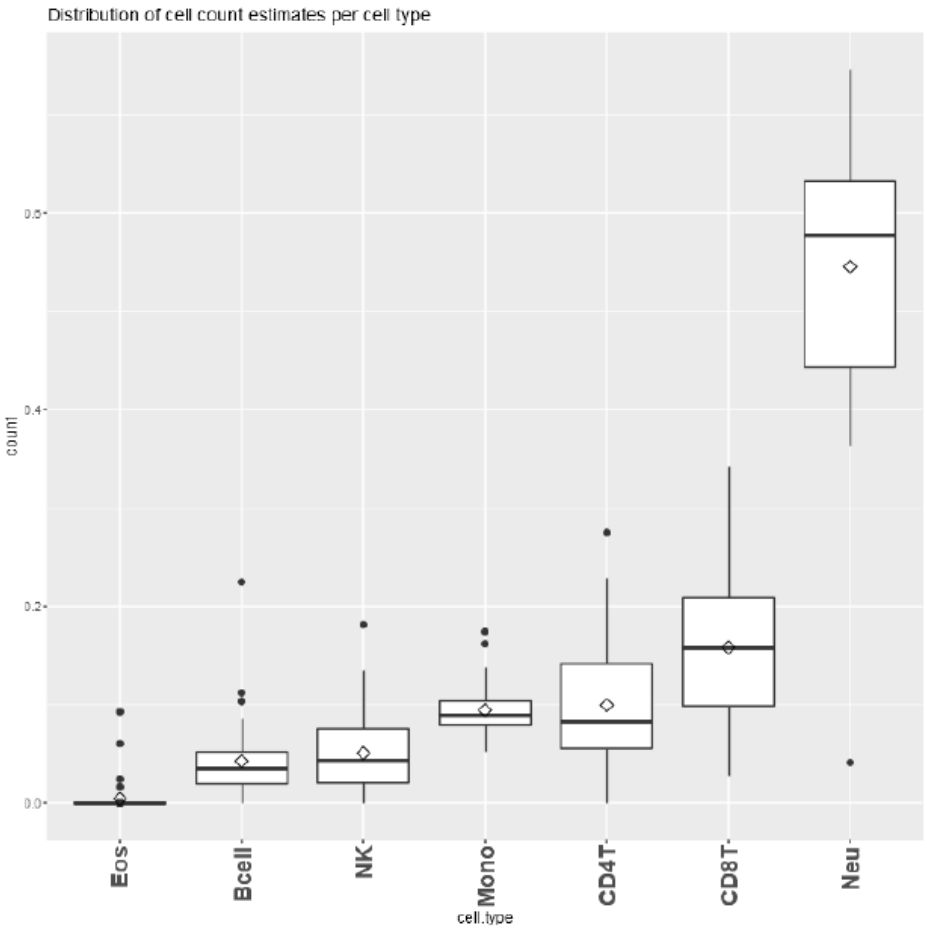
Supplementary Figures 11a-e: Forrest plots representing regression coefficients and 95% confidence intervals (*x*-axis) after functional normalisation for each slide (bar 1 to 6) and each row (bar 7 to 14) divided into five figures representing principal component 1 (PC1) (Supplementary Figure 11a), PC2 (Supplementary Figure 11b), PC3 (Supplementary Figure 11c), PC4 (Supplementary Figure 11d) and PC5 (Supplementary Figure 11e).

Surrogate variable analysis

Since all batch effects were not be fully removed using functional normalisation, we also performed surrogate variable analysis (SVA) [41]. In addition to addressing the variance explained by batch effects and other technical sources, SVA can also account for unknown unwanted variance caused by biological differences in the samples which are unrelated to the phenotype of interest e.g. covariates such as age, body mass index (BMI), smoking, HIV status, medication use, childhood trauma exposure, lifetime trauma exposure, alcohol use and depression may have an impact on methylation levels irrespective of PTSD status [41,42]. The resulting surrogate variables were included as covariates in EWAS models.

Blood cell type composition

Blood cell type composition is another source of bias often introduced when using whole blood to investigate methylation profiles. Whole blood contains several different cell types and each type has its own methylation profile [43]. SVA has been shown to be successful in removing the unwanted variance introduced by cell-type distribution, but as a precautionary measure we also estimated cell type composition using the Houseman algorithm and the publicly available blood cell type reference dataset GSE35069 (available on the Gene Expression Omnibus) [44] and included cell type compositions for B lymphocytes, CD4 T-cells, CD8 T-cells, eosinophils, monocytes, neutrocytes and natural killer cells in the final analysis (see Supplementary Figure 12 for estimated cellular composition).



Supplementary Figure 12: Boxplots illustrating the estimated cellular composition for each reference cell type across all samples. The *x*-axis represents the cell type (eosinophils, B lymphocytes, natural killer cells, monocytes, CD4 T-cells, CD8 T-cells and neutrophils). The *y*-axis represents the estimated cell proportions.

Differentially methylated regions (DMRs)

Differentially methylated regions (DMRs) were identified using the *dmrff* R package [45]. DMR analysis improves statistical power given that the EWAS CpG sites can be subdivided into clusters and associations assessed as a group rather than individually. There are often strong dependencies between CpG sites, especially those clustering around the same gene or those in close genomic proximity to each other [45,46]. The default setting applied by the *dmrff* package for qualification as a DMR was used in this study with the exception of the distance between CpG sites. The default value in the *dmrff* package is 500 bp apart, but we applied a more stringent criteria of 100 bp apart since CpG sites are more likely to co-methylate if they are in closer proximity to each other [47,48].

The more conservative Bonferonni adjustment was used to correct for multiple testing in the DMR analysis as opposed to false discovery rate (FDR), which was used in the differentially methylated position (DMP) analysis [49]. FDR was deemed suitable for the DMP analysis since intercorrelation of CpG sites on the MethylationEPIC array is highly likely and a Bonferonni correction (especially when several thousands of tests are conducted at once) would most likely result in inflated false negatives (type 2 errors) [50,51]. FDR reduces false positives (type 1 errors) by adjusting *p*-values according to their number ranking (ranked from most significant/smallest p-value to least significant/largest p-value) and are less stringent compare to a Bonferonni correction which defines the new significance level as $p < .05$ / by the number of tests completed [49,52,53].

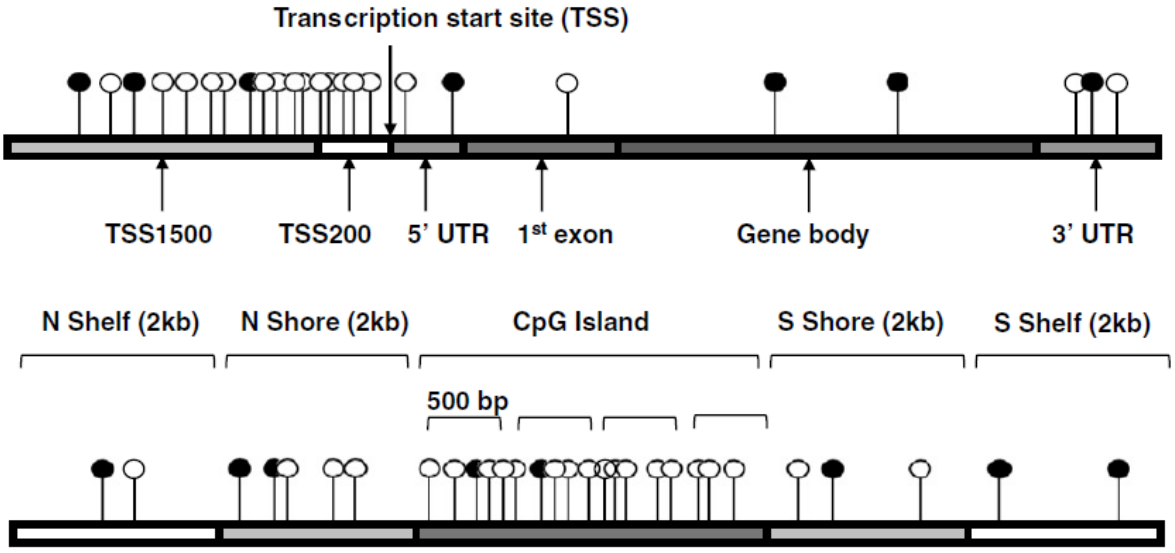
A Bonferroni correction was deemed appropriated for the DMP analysis because the it assumes that each test is independent of the others, which is more likely to be true in the DMR analysis since it identifies CpGs covarying within a region in an individual gene [46]. A Bonferroni correction was also deemed appropriate for the DMR analysis since it is a confirmative approach rather than an explorative approach like the DMP analysis and more stringent criteria are often applied in confirmative analyses [45,50].

Annotation

Coordinates resulting from the DMP and DMR analyses were annotated using the MethylationEPIC_v-1-0_B4 manifest. All genomic coordinates reported in this study is in reference to the Hg19/GRCh37 human genome assembly. The location of a CpG site was determined using the 'University of California Santa Cruz (UCSC) reference gene group' (see Supplementary Figure 13). TSS200 indicates that the CpG site is located 0 to 200 bp upstream of the transcription start site (TSS), TSS1500 indicates that the CpG site is located 200-1500bp

upstream of the TSS, 5'UTR indicates that the CpG site is located within the 5' untranslated region between the TSS and ATG (the protein coding start site), body indicates that the CpG site is located between the ATG and the stop codon (protein coding end site), irrespective of introns, exons, TSS or promoters in the region, 3'UTR indicates that the CpG site is located between the stop codon and the polyadenylation signal. The 'regulatory feature group' provided by the Methylation Consortium was used to determine if a CpG site is located in a regulatory sequence (region associated with increasing or decreasing expression of genes). CpG sites located in a regulatory sequence are labelled as promoter associated [34].

The location of individual CpG sites in relation to a CpG island in the gene were determined using the 'UCSC CpG island location classification' (see Supplementary Figure 13). A CpG island was defined as a region with more than 50% of the nucleotide sequence consisting of CpG sites and at least 200pb in length. A CpG island shore was defined as a region 0 to 2000bp from a CpG island and a CpG island shelf was defined as a region 2000 to 4000 bp from the island. North (N) and South (S) indicates the direction of the shore/shelf with N indicating the shore/shelf is upstream (5') form the CpG island and S indicating the shore/shelf is downstream (3') from the CpG island. OpenSea indicates that the region the CpG site is located in is not in a CpG island or a shelf/shore of the island.



Supplementary Figure 13: Region in a gene in which a CpG site is located according to the University of California Santa Cruz (UCSC) reference gene group. Location of an individual CpG sites in relation to a CpG island in the gene according to UCSC CpG Island location classification. Reprinted from "High density DNA methylation array with single CpG site resolution" by M Bibikova, B Barnes, C Tsan, V Ho, B Klotzle, JM Le, D Delano, L Zhang, GP Schroth, KL Gunderson, JB Fan, R Shen, 2011, *Genomics*, *98*, p. 291, Copyright 2011 by Elsevier Incorporated [34].

Validation, replication and longitudinal analysis

Sample concentrations were normalised to 50 ng/ul and shipped on ice to Inqaba Biotec in Pretoria, South Africa for bisulfite conversion and analysis using EpiTYPER Sequenom MassARRAY (Agena Bioscience, California, United States) technology. The method involved: (1) PCR primer design using Sequenom Epidesigner software (www.epidesigner.com); (2) bisulfite conversion using the Zymo EZ DNA methylation kit (Zymo Research, California, United States); (3) PCR amplification of the target region using t7-promoter tagged primers; (4) RNA transcription and base-specific RNA cleavage; (5) quantification of DNA methylation based on the size and mass of RNA fragments using the SpectroCHIP Array, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and EpiTYPER software. The samples were randomly assigned to one of three plates (96 samples per plate). The EpiTYPER procedure is highly reproducible and has been found to detect methylation levels as low as 5% [89,90].

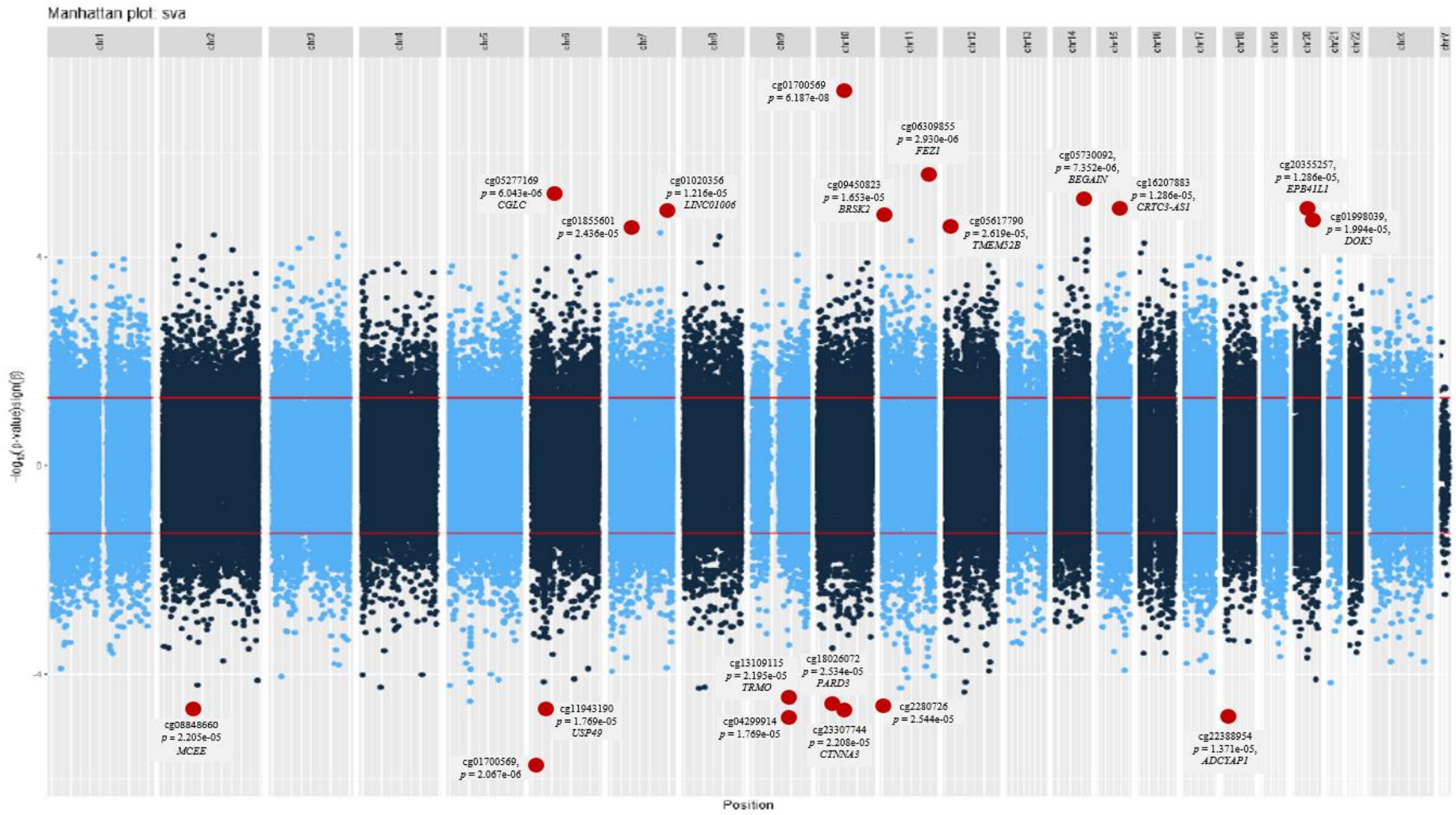
The primer designs used in the EpiTYPER analysis for *BRSK2* and *ADCYAP1* are provided below:

BRSK2: forward primer, 5'-aggaagagagGAAGGAGTTTGTTAGGGTGTAGGAG-3' and reverse primer, 5'- cagtaatacgactcactatagggagaaggctAAACAAAACCAATCTCTACCTTAAA-3'

ADCYAP1: forward primer, 5'- aggaagagagGGTTTTTTGAGTTGAATAGTATTTGG-3' and reverse primer, 5'- cagtaatacgactcactatagggagaaggctTCCTACCTAACAACTCTCCTAACAA-3'

RESULTS

Additional results are presented in supplementary figures 14-17 and supplementary tables 3-14.



Supplementary Figure 14: Manhattan plot indicating the top twenty CpG sites significantly associated with PTSD prior to correction for multiple testing ($p < .05$). CpG sites are organised according to their chromosomal position on the horizontal axis. CpG sites above the upper red line indicates that the site showed increased methylation in the group without PTSD and CpG sites below the lower red line indicates that the site showed decreased methylation in the group without PTSD

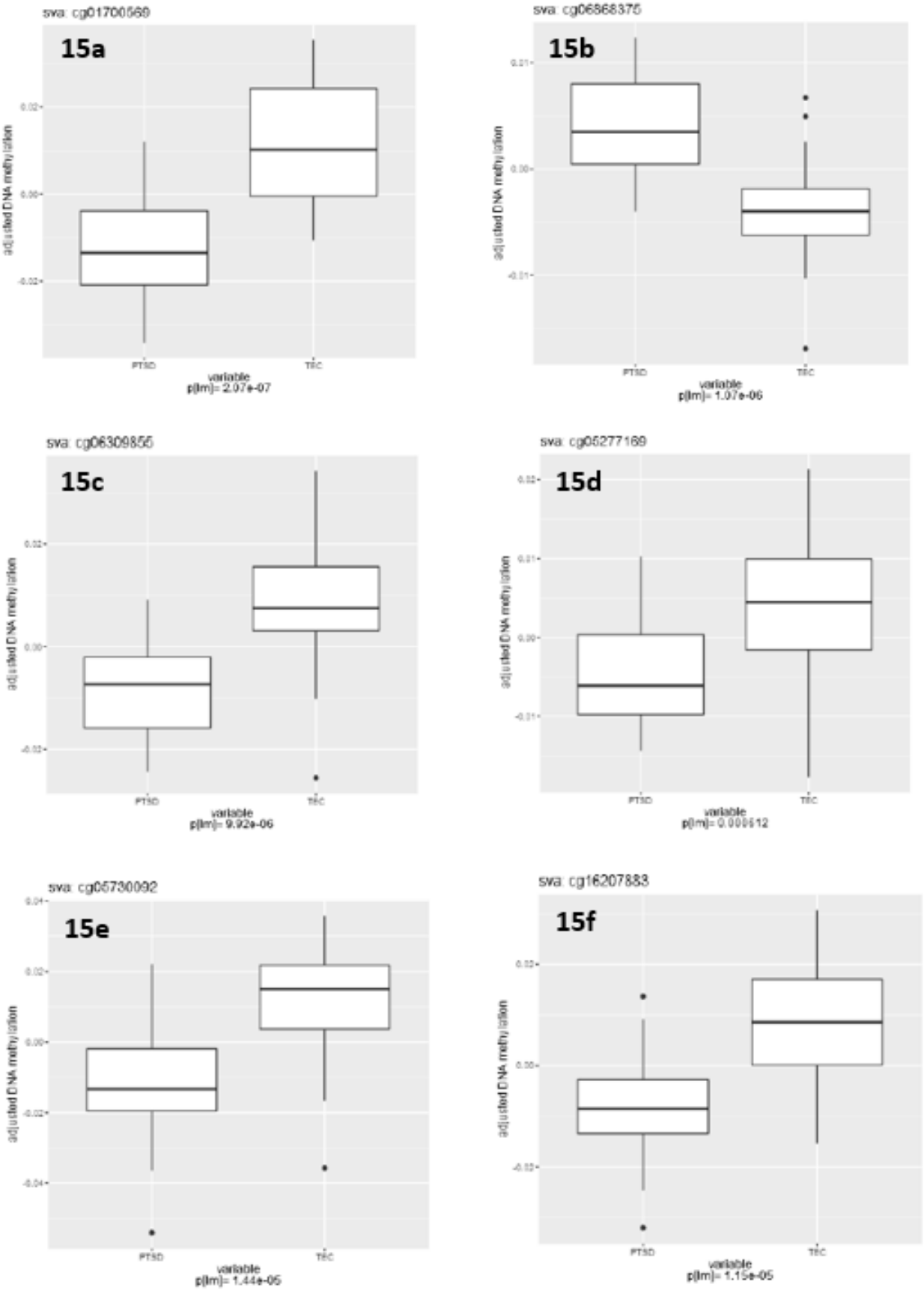


Figure 15a-t: Boxplots illustrating the difference in methylation levels between participants with and without PTSD at 3-months post-rape for the top twenty CpG sites resulting from the epigenome-wide association study.

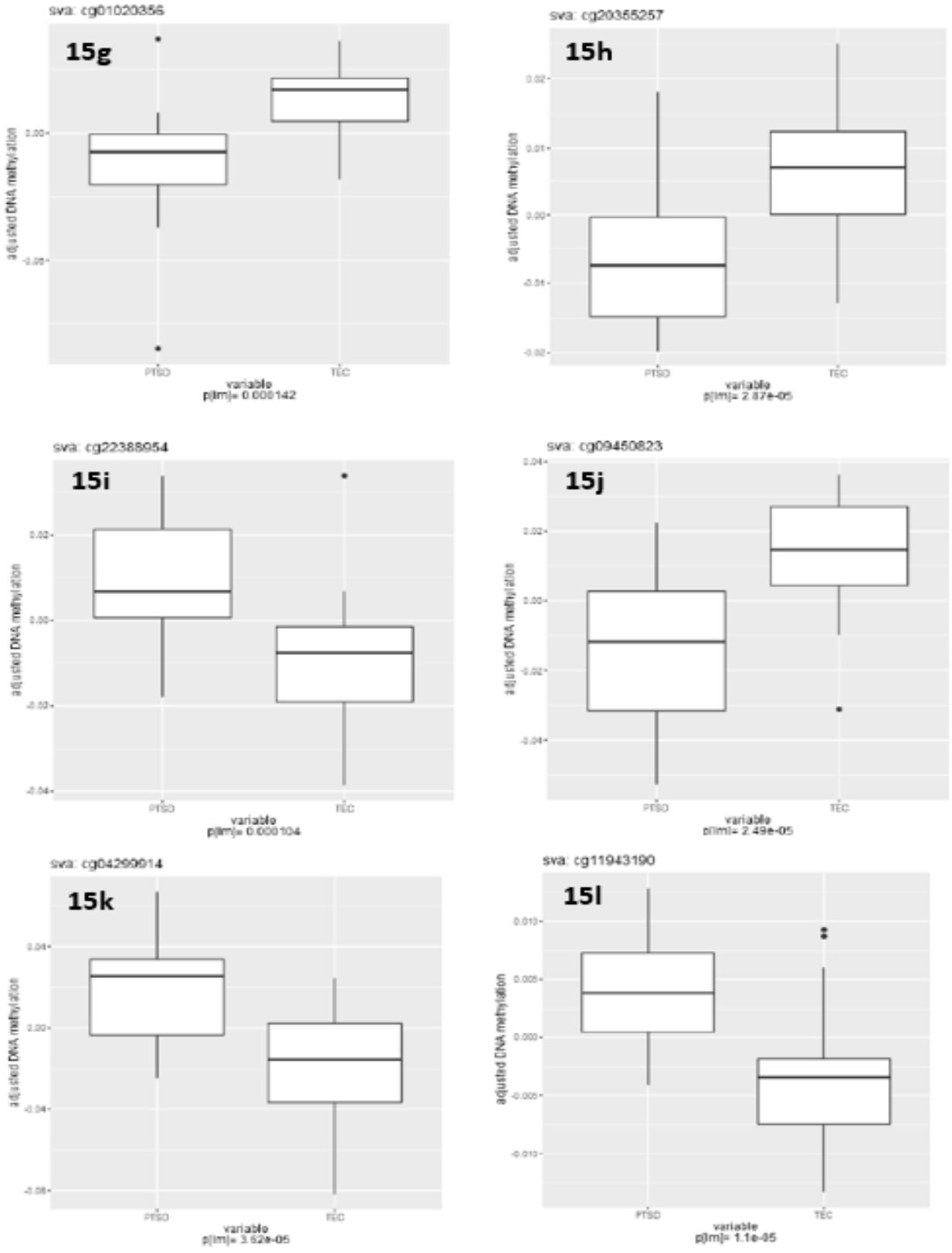


Figure 15a-t continued: Boxplots illustrating the difference in methylation levels between participants with and without PTSD at 3-months post-rape for the top twenty CpG sites resulting from the epigenome-wide association study.

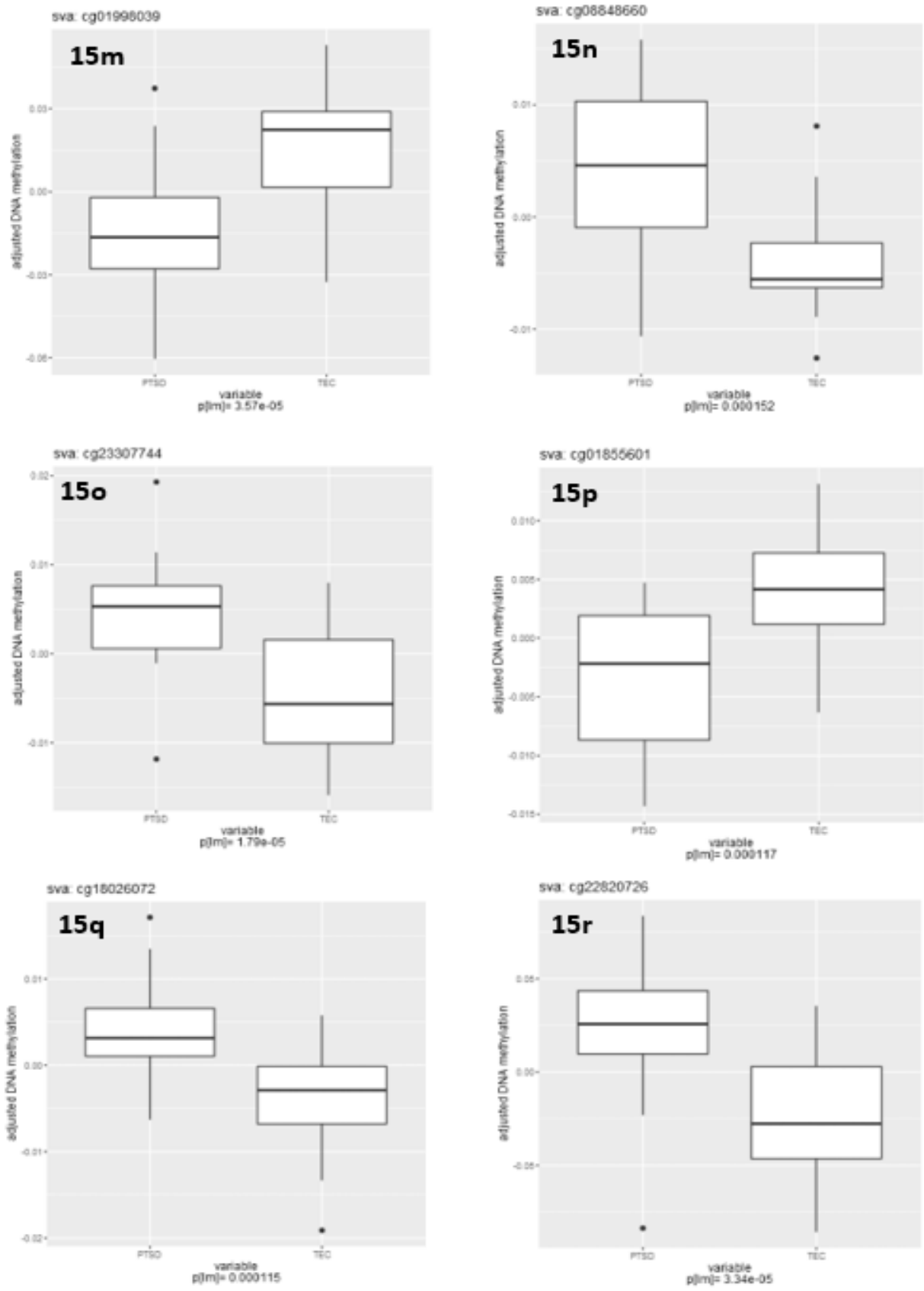


Figure 15a-t continued: Boxplots illustrating the difference in methylation levels between participants with and without PTSD at 3-months post-rape for the top twenty CpG sites resulting from the epigenome-wide association study.

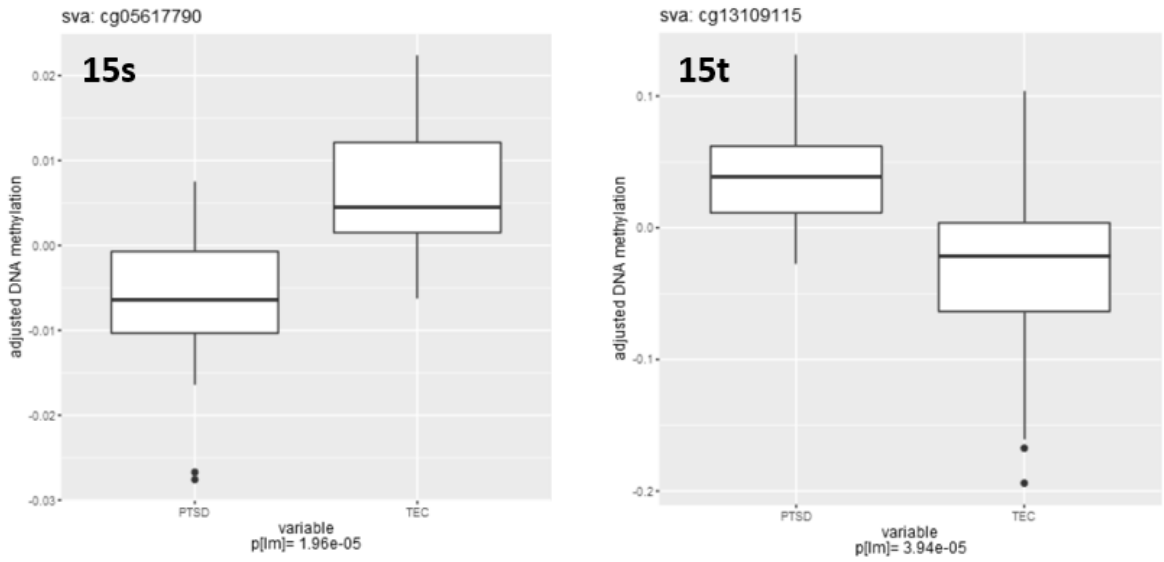


Figure 15a-t continued: Boxplots illustrating the difference in methylation levels between participants with and without PTSD at 3-months post-rape for the top twenty CpG sites resulting from the epigenome-wide association study.

Supplementary Table 3: *Genome-wide differentially methylated positions (DMPs) and regions (DMRs) associated with PTSD*

Gene Name[1]	Position[2]	Probe	Relation to Island[3]	Location in Gene[3,4]	*β*	SE	*t/z*	*p*	Adj. *p*	Other exposures/ phenotypes associated with the CpG site[5]
Differentially methylated positions (DMPs)										
NA, *SLC16A9*[6]	Chr10:61385771	cg01700569	OpenSea	NSF	0.031	0.004	7.119	6.187e-08	0.049233	None
NA, *IRF4*[6]	Chr6:429318	cg06868375	OpenSea	NSF	-0.009	0.002	-5.846	2.067e-06	0.777284	None
FEZ1	Chr11:125365803	cg06309855	Island	5'UTR	0.022	0.004	5.727	2.930e-06	0.777284	Gestational age
CGLC	Chr6:53371893	cg05277169	OpenSea	Body	0.013	0.002	5.468	6.043e-06	0.999998	None
BEGAIN	Chr14:101036470	cg05730092	S_Shore	TSS1500	0.032	0.006	5.401	7.352e-06	0.999998	Gestational age
CRTC3-AS1	Chr15:91193536	cg16207883	OpenSea	Body	0.021	0.004	5.233	1.183e-05	0.999998	None
LINC01006	Chr7:156267149	cg01020356	OpenSea	Body	0.025	0.005	5.224	1.216e-05	0.999998	Ethnicity; osteonecrosis of the femoral head
EPB41L1	Chr20:34818105	cg20355257	OpenSea	3'UTR	0.017	0.003	5.204	1.286e-05	0.999998	Down syndrome
ADCYAP1	Chr18:905177	cg22388954	Island	5'UTR; TSS200	-0.025	0.005	-5.181	1.371e-05	0.999998	B acute lymphoblastic leukemia
BRSK2	Chr11:1431833	cg09450823	Island	Body	0.036	0.007	5.115	1.653e-05	0.999998	None
NA, *MIR4290*[6]	Chr9:92782655	cg04299914	OpenSea	NSF	-0.049	0.010	-5.091	1.769e-05	0.999998	None
USP49	Chr6:41790313	cg11943190	OpenSea	5'UTR	-0.010	0.002	-5.050	1.970e-05	0.999998	Rheumatoid arthritis; exercise
DOK5	Chr20:53127455	cg01998039	OpenSea	5'UTR; Body	0.040	0.008	5.049	1.994e-05	0.999998	Down syndrome; aging
MCEE	Chr2:71338735	cg08848660	OpenSea	Body	-0.011	0.002	-5.012	2.205e-05	0.999998	None

CTNNA3	Chr10:68940214	cg23307744	OpenSea	Body	-0.012	0.002	-5.012	2.208e-05	0.999998	None
NA, *AC004854.2*[6]	Chr7:44965393	cg01855601	OpenSea	NSF	0.010	0.002	4.975	2.436e-05	0.999998	None
PARD3	Chr10:35016204	cg18026072	OpenSea	Body	-0.009	0.002	-4.961	2.534e-05	0.999998	None
NA, *BRSK2*[6]	Chr11:1401914	cg22820726	N_Shelf	NSF	-0.063	0.013	-4.963	2.544e-05	0.999998	None
TMEM52B	Chr12:10322100	cg05617790	OpenSea	Body; TSS1500	0.015	0.003	4.953	2.619e-05	0.999998	None
TRMO	Chr9:100662939	cg13109115	OpenSea	NSF	-0.103	0.021	-4.915	2.915e-05	0.999998	None

Differentially methylated regions (DMRs)

LRRC34	Chr3:169530817-169530920				0.071	0.007	9.631	5.910e-22	4.897e-16	
		cg16024214[7]	S_Shore	TSS1500						None
		cg27467234[7]	S_Shore	TSS1500						None
		cg04714994[7]	S_Shore	TSS1500						Ethnicity
		cg05344026[7]	S_Shore	TSS1500						Ethnicity
LRRC34	Chr3:169531663-169531864				0.091	0.010	9.101	8.960e-20	7.424e-14	
		cg12324144[7]	S_Shore	TSS1500						None
		cg03369965[7]	S_Shore	TSS1500						Follicular thyroid carcinoma; type 2 diabetes; ethnicity
		cg13337095[7]	S_Shore	TSS1500						None
		cg10994914[7]	S_Shore							B Acute lymphoblastic leukemia
PLD6	Chr17:17109640-17109817				0.026	0.003	9.316	1.214e-20	1.006e-14	

		cg03292213[7]	Island	5'UTR						Ankylosing spondylitis
		cg11392858[7]	Island	TSS200						Ankylosing spondylitis
		cg23661344[7]	Island	TSS200						Ankylosing spondylitis
		cg26539818[7]	Island	TSS200						Ankylosing spondylitis
		cg00572586[7]	N_Shore	TSS200						Juice consumption
		cg14099398[7]	N_Shore	TSS200						None
		cg02880176[7]	N_Shore	TSS200						None
NA, *LOC101928343*[6]	Chr17:72595585-72595587				0.093	0.011	8.166	3.191e-16	2.644e-10	
		cg01238395	OpenSea	NSF						None
		cg05192716	OpenSea	NSF						None
SLC38A11	Chr2:165811982-165812244				-0.022	0.003	-7.718	1.178e-14	9.762e-09	
		cg03464655	OpenSea	5'UTR						Adrenocortical carcinoma; Nicolaides Baraitser syndrome (NCBRS); neurodevelopmental presentations and congenital anomalies; Gulf War illness
		cg20641955	OpenSea	TSS200						None
		cg16376155	OpenSea	TSS200						None
		cg05339727	NSF	TSS200						Age; sex; ethnicity; *SETD1-B*-related syndrome
		cg05090759	OpenSea	TSS200						Clear cell renal carcinoma; sex; *SETD1-B*-related syndrome

		cg12301695	OpenSea	TSS200						Oral squamous cell carcinoma; colorectal cancer; *SETD1-B*-related syndrome; Gulf War illness
		cg12753297	OpenSea	TSS1500						None
CC2D2A	Chr4:15471214-15471399				0.015	0.002	7.856	3.964e-15	3.285e-09	
		cg21329975	OpenSea	TSS1500						Smoking status; air population exposure; gestational age
		cg16509355	OpenSea	TSS1500						Fetal vs adult liver; smoking status; gestational age; Down syndrome
		cg21123203	NSF	NSF						Smoking status; fruit consumption
		cg02964094	OpenSea	TSS200						Gingivobuccal oral squamous cell carcinoma; Gulf War illness
		cg18470593	OpenSea	TSS200						Fetal vs adult liver; smoking status; obesity
		cg20184469	OpenSea	TSS200						None
ESM1	Chr5:54281478-54281733				-0.042	0.004	-9.746	9.935e-13	8.233e-07	
		cg21180956	OpenSea	TSS200						None
		cg13106512	OpenSea	TSS200						Myalgic encephalomyelitis / chronic fatigue syndrome
		cg24403549	OpenSea	TSS200						Myalgic encephalomyelitis / chronic fatigue syndrome; ethnicity
		cg10631947	OpenSea	TSS1500						Height

		cg20059697	OpenSea	TSS1500						Down syndrome
		cg16462183	OpenSea	TSS1500						Gestational age; Down syndrome; hepatocellular carcinoma; colorectal laterally spreading tumour; perinatally-acquired HIV
SORBS2	Chr4:186732837-186733060				0.047	0.006	7.452	9.180e-14	7.607e-08	
		cg12309668	OpenSea	5'UTR; TSS1500						Down syndrome; early childhood development
		cg04392082	OpenSea	5'UTR; TSS1500						Down syndrome; gestational age
		cg02790305	OpenSea	5'UTR; TSS1500						Down syndrome; aging; gestational age
		cg26785346	NSF	NSF						Down syndrome; early childhood development gestational age
		cg01933073	OpenSea	5'UTR; TSS1500						Down syndrome; gestational age
		cg18342119	NSF	NSF						Down syndrome; aging
		cg13921444	OpenSea	5'UTR; TSS1500						Down syndrome; aging; respiratory allergies; early childhood development; gestational age
		cg04348265	OpenSea	5'UTR; TSS1500						Down syndrome; aging; early childhood development
ANKRD33	Chr12:52281674-52282079				0.030	0.004	7.499	6.442e-14	5.338e-08	
		cg12053065	OpenSea	TSS200						None
		cg19974223	OpenSea	TSS200						None
		cg09755932	OpenSea	TSS200						None

		cg10384244	OpenSea	TSS200						None
		cg19948393	OpenSea	5'UTR						Down syndrome
		cg26279928	OpenSea	5'UTR						None
		cg04349021	OpenSea	5'UTR						None
		cg17424856	OpenSea	5'UTR						None
		cg03110082	OpenSea	5'UTR						None
COL18A1	Chr21:46875372-46875500				0.029	0.004	7.373	1.671e-13	1.385e-07	
		cg24201814	N_Shore	TSS200; Body						None
		cg06552850	N_Shore	TSS200; Body						None
		cg07279557	N_Shore	TSS200; Body						Down syndrome; fetal vs adult liver; early childhood development; gestational age; maternal smoking; sex; neurodevelopmental presentations and congenital anomalies
		cg04401043	N_Shore	TSS200; Body						None
		cg11029358	N_Shore	5'UTR; Body						Gestational age; neurodevelopmental presentations and congenital anomalies
		cg07641662	N_Shore	Body						None
BRSK2	Chr11:1463541-1463670				0.112	0.016	7.131	9.935e-13	8e-07	
		cg12186219	N_Shore	Body						Childhood stress; ethnicity
		cg14064268	N_Shore	Body						Aging; childhood stress; ethnicity

		cg10590925	N_Shore	Body						Aging, childhood stress, ethnicity
		cg17429870	N_Shore	Body						Aging, childhood stress, ethnicity
		cg18651858	N_Shore	Body						Ethnicity
AC004895.4	Chr7:6120483-6120572				-0.053	0.008	-6.864	6.686e-12	5.5e-06	
		cg27459591[7]	N_Shore	NSF						Down syndrome
		cg10437320[7]	N_Shore	NSF						Down syndrome
		cg07374086[7]	N_Shore	NSF						Down syndrome
		cg14614937[7]	N_Shore	NSF						None
SPON1	Chr11:13983705-13983989				-0.021	0.003	-6.857	7.040e-12	5.8e-06	
		cg08309747	N_Shore	TSS1500						None
		cg26255604	N_Shore	TSS1500						None
		cg12085698	N_Shore	TSS200						Pancreatic ductal adenocarcinoma; gestational age; inflamed Crohn's disease
		cg25486824	N_Shore	TSS200						Pancreatic ductal adenocarcinoma; gestational age; sex; inflamed Crohn's disease
		cg11028624	N_Shore	TSS200						Pancreatic ductal adenocarcinoma; gestational age; inflamed Crohn's disease
		cg22805485	N_Shore	TSS200						Pancreatic ductal adenocarcinoma; gestational age; maternal smoking; inflamed Crohn's disease

		cg24258347	N_Shore	TSS200						Pancreatic ductal adenocarcinoma; gestational age; aging; preterm birth; maternal hypertensive disorder; inflamed Crohn's disease
		cg10153349	N_Shore	TSS200						Gestational age; sex; prenatal paracetamol exposure; aging; inflamed Crohn's disease
		cg09191626	N_Shore	5'UTR						Gestational age; maternal smoking; follicular thyroid carcinoma
		cg02394317	N_Shore	TSS200						None
ESM1	Chr5:54281198-54281362				-0.041	0.006	-6.827	8.662e-12	7.2e-06	
		cg14972155	OpenSea	NSF						Italian ethnicity; sex; maternal plasma folate; chronic obstructive pulmonary disease
		cg06837426	OpenSea	NSF						Maternal plasma folate
		cg20451680	OpenSea	NSF						Chronic obstructive pulmonary disease; type 2 diabetes
		cg20673840	OpenSea	5'UTR						Chronic obstructive pulmonary disease; type 2 diabetes
ADCYAP1	Chr18:905177-905180				-0.022	0.003	-6.761	1.370e-11	1.13e-05	
		cg22388954	Island	5'UTR; TSS200						B Acute lymphoblastic leukemia
		cg11773720	Island	5'UTR; TSS200						None
INPP5A	Chr10:134549665-134549800				0.016	0.002	6.700	2.081e-11	1.72e-05	B Acute lymphoblastic leukemia
		cg18305652	Island	Body						B Acute lymphoblastic leukemia
		cg16311946	Island	Body						B Acute lymphoblastic leukemia

		cg11624078	Island	Body						None
C21orf62	Chr21:34185960-34186122				0.021	0.003	6.345	2.219e-10	1.839e-4	
		cg03803086	OpenSea	5'UTR						Fetal vs adult liver; early childhood development; gestational age; sex; air pollution; body mass index; Down syndrome; follicular thyroid carcinoma; thyroid lesion
		cg27427104	OpenSea	5'UTR						Werner syndrome; Gulf War illness
		cg00780604	OpenSea	5'UTR						Gulf War illness
		cg23163200	OpenSea	TSS200						Fetal vs adult liver; early childhood development; gestational age; multiple sclerosis; Werner syndrome
LRRC34	Chr3:169530621-169530692				0.050	0.008	6.184	6.268e-10	0.001	
		cg02860240[7]	S_Shore	TSS1500						Hepatocellular carcinoma; ethnicity
		cg24655016[7]	S_Shore	TSS200						Ethnicity
		cg23707559[7]	S_Shore	TSS200						None
RP11-1085N6.3	Chr14:57197389-57197433				0.023	0.004	6.146	7.946e-10	0.001	
		cg10247374	OpenSea							None
		cg11668797	OpenSea							None
		cg08934402	OpenSea							Ethnicity
TSPEAR-AS2	Chr21:45937342-45937406				0.036	0.006	6.115	9.646e-10	0.001	
		cg05005821	OpenSea	Body						None

		cg04289880	OpenSea	Body						Sex; maternal alcohol consumption
NA, *SFXN3*[6]	Chr10:102806871-102806933				0.042	0.007	6.029	1.650e-09	0.001	
		cg04270401	N_Shore							Fetal vs adult liver; early childhood development; gestational age; alcohol consumption; aging; follicular thyroid carcinoma; obesity
		cg24067118	N_Shore							Fetal vs adult liver; early childhood development; gestational age; alcohol consumption; absolute fat mass; aging; body mass index; fat free mass index; fat mass index
MIR125B1	Chr11:121970694-121970768				0.026	0.004	5.996	2.019e-09	0.002	
		cg02101355	OpenSea	Body; TSS200						Clear cell renal carcinoma; psoriasis
		cg03891346	NSF	NSF						Clear cell renal carcinoma
		cg24213115	OpenSea	Body; TSS200						Down syndrome
		cg07281370	OpenSea	TSS1500; Body						Clear cell renal carcinoma; psoriasis
NA, *LOC 343052*[6]	Chr1:153762359-153762434				0.019	0.003	5.975	2.301e-09	0.002	
		cg18042081	OpenSea	NSF						None
		cg13502252	OpenSea	NSF						Gestational age; fetal alcohol spectrum disorder
		cg11710659	OpenSea	NSF						Clear cell renal carcinoma; rheumatoid arthritis; maternal smoking; gestational age
NA, *LOC 343052*[6]	Chr1:153762201-153762244				0.025	0.004	5.881	4.068e-09	0.003	

		cg26182859	OpenSea	NSF						Fetal vs adult liver; early childhood development; gestational age; smoking status; sex; acute myelocytic leukemia; aging; Crohn's disease
		cg09762021	OpenSea	NSF						Smoking status; gestational age
		cg12067764	OpenSea	NSF						Gestational age; aging
C5orf66-AS1	Chr5:134375885-134375904				0.011	0.002	5.829	5.589e-09	0.005	
		cg11469026	Island	TSS200; 5'UTR						None
		cg12887021	Island	TSS200; 5'UTR						None
FSCN2	Chr17:79504142-79504180				0.006	0.001	5.820	5.874e-09	0.005	
		cg07707031	Island	3'UTR						HIV infection
		cg00333483	Island	NSF						None
FOXJ3	Chr1:42801728-42801761				-0.020	0.004	-5.734	9.805e-09	0.008	
		cg12375158	S_Shore	TSS1500; TSS200						*SETD1B*-related syndrome
		cg04049626	NSF	NSF						*SETD1B*-related syndrome
		cg18174678	S_Shore	TSS1500						Fetal vs adult liver; smoking status; maternal smoking; gestational age; *SETD1B*-related syndrome; Gulf War illness
SLC39A13	Chr11:47430791-47430812				-0.014	0.003	-5.693	1.250e-08	0.010	
		cg05778943	S_Shore	5'UTR						None
		cg19668951	S_Shore	5'UTR						Fetal vs adult liver; gestational age; sex; multiple sclerosis

TPPP	Chr5:672845-672910				0.054	0.009	5.685	1.305e-08	0.011	
		cg22879098	OpenSea	Body						Fetal vs adult liver; maternal smoking
		cg24082121	OpenSea	Body						Fetal vs adult liver; maternal smoking; aging
		cg10986412	OpenSea	Body						Maternal smoking; fruit consumption; aging
LINC01529	Chr19:36288702-36288895				0.030	0.005	5.577	2.450e-08	0.020	
		cg02456406	OpenSea							Rheumatoid arthritis
		cg14624451	OpenSea	Body						None
		cg11791444	OpenSea	Body						None
		cg09182728	OpenSea	TSS200						None
C5orf63	Chr5:126408756-126408806				-0.052	0.009	-5.535	3.115e-08	0.026	
		cg14340928[7]	N_Shore	5'UTR						Fetal vs adult liver
		cg17848407[7]	N_Shore	5'UTR						None
HCCA2	Chr11:1769382-1769462				0.036	0.007	5.465	4.632e-08	0.038	
		cg08269485	Island	Body; TSS200						Early childhood development; prostate cancer
		cg03846408	Island	Body; TSS200						Early childhood development; prostate cancer
		cg08966208	Island	Body; TSS200						Early childhood development; preterm birth; prostate cancer

		cg02916272	Island	Body; TSS200						Early childhood development; gestational age; preterm birth; prostate cancer
		cg20272155	Island	Body; TSS200						Early childhood development; gestational age
OXT	Chr20:3052058-3052262				-0.030	0.005	-5.452	4.973e-08	0.041	
		cg19776589	N_Shore	TSS1500						None
		cg07747220	Island	TSS200						Early childhood development; gestational age; aging; childhood stress; multiple sclerosis; preterm birth; antiphospholipid syndrome; neurodevelopmental presentations and congenital anomalies; *SETD1B*-related syndrome
		cg16887334	Island	TSS200						Aging; sex; childhood stress; short-term diesel exhaust inhalation
		cg13285174	Island	TSS200						Early childhood development; gestational age; aging; cleft palate cs cleft lip; HIV infection; childhood stress; multiple sclerosis; preterm birth; Alzheimer's disease; *SETD1B*-related syndrome
		cg26267561	Island	TSS200						Gestational age; cleft palate vs cleft lip; aging; childhood stress multiple sclerosis; preterm birth; epithelial ovarian cancer; *SETD1B*-related syndrome; Gulf War illness
		cg01644611	Island	TSS200						Early childhood development; gestational age; cleft palate vs cleft lip; aging; childhood stress; multiple sclerosis; preterm birth;

										neurodevelopmental presentations and congenital anomalies
		cg13725599	Island	TSS200						Early childhood development; gestational age cleft palate vs cleft lip; aging; childhood stress
ZNF595	Chr4:53010-53109				0.018	0.003	5.424	5.830e-08	0.048	
		cg01419539	N_Shore	TSS1500						Early childhood development; smoking status; aging; alcohol consumption
		cg07697276	NSF	NSF						Early childhood development; alcohol consumption
		cg26385085	N_Shore	TSS200						Early childhood development

[1] Identified using the GENECODE database; [2] identified using the Human Genome 19 (HG19) build from the Genome Reference Consortium; [3] identified using the University of California Santa Cruz (UCSC) Genomic Institute/Genome Browser; [4] multiple listings indicate splice variants; [5] identified using the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) catalog of epigenome-wide association studies[54] and the China National Center for Bioinformation National Genomics Data Center epigenome-wide association studies (EWAS) atlas[55]; [6] CpG sites located in a region not attributed to a gene, the gene closest to the CpG site is provided; [7] The site is associated with a promoter region - identified using the Methylation Consortium project.

Abbreviations: Standard error (SE); adjusted (Adj); not applicable (NA); solute carrier family 16 member 9 *(SLC16A9)*; not specified (NSF); interferon regulatory factor 4 *(IRF4)*; fasciculation and elogation protein zeta 1 *(FEZ1)*; 5' untranslated region (5'UTR); glutamate-cysteine ligase catalytic subunit (*GCLC);* brain-enriched guanylate kinase-associated protein *(BEGAIN)*; transcription start site 1500 (TSS1500); South shore (S_Shore); CRTC3 antisense RNA 1 (*CRTC3-AS1)*; long intergenic non-protein coding RNA 1006 *(LINC01006);* erythrocyte membrane protein band 4.1 Like 1 *(EPB41L1)*; 3' untranslated region (3'UTR); adenylate cyclase activating polypeptide 1 *(ADCYAP1)*; transcription start site 200 (TSS200); brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; microRNA 4290 *(MIR4290)*; ubiquitin specific peptidase 49 *(USP49)*; docking protein 5 *(DOK5)*; methylmalonyl-CoA epimerase *(MCEE)*; catenin alpha 3 *(CTNNA3)*; novel transcript antisense to purine rich element binding protein B *(AC004854.2)*; PAR-3 family cell polarity regulator (*PARD3)*; North shelf (N_Shelf); transmembrane protein 52B *(TMEM52B)*; TRNA methyltransferase O (*TRMO);* leucine-rich repeat-containing protein 34 *(LRRC34)*; phospholipase D family member 6 *(PLD6)*; North shore

(N_Shore); solute carrier family 38 member 11 *(SLC38A11)*; coiled-coil and C2 domain-containing protein 2A *(CC2D2A)*; endothelial cell specific molecule 1 *(ESM1)*; sorbin and SH3 domain-containing protein 2 *(SORBS2)*; ankyrin repeat domain-containing protein 33 *(ANKRD33)*; collagen type XVIII alpha 1 chain *(COL18A1)*; spondin 1 *(SPON1)*; inositol polyphosphate-5-phosphatase A *(INPP5A)*; chromosome 21 open reading frame 62 *(C21orf62)*; thrombospondin type laminin G domain and EAR repeats antisense RNA 2 *(TSPEAR-AS2)*, microRNA 125b-1 *(MIR125B1)*; chromosome 5 open reading frame 66 antisense RNA *(C5orf66AS1);* fascin actin-bundling protein 2 *(FSCN2)*; forkhead box J3 *(FOXJ3)*; solute carrier family 39 member 13 *(SLC39A13)*; tubulin polymerization promoting protein *(TPPP)*; long intergenic non protein coding RNA 1529 *(LINC01529)*; chromosome 5 open reading frame 63 *(C5orf63)*; hepatocellular carcinoma-associated gene 2 *(HCCA2)*, oxytocin/neurophysin I prepropeptide *(OXT)*; and zinc finger protein 595 *(ZNF595)*

Blood-brain methylation comparison

Co-variation in methylation levels between blood and brain tissue was explored using the online Blood Brain DNA Methylation Comparison Tool [56]. Blood methylation of the *BRSK2* CpG sites were highly correlated with methylation in the prefrontal cortex, superior temporal gyrus and the cerebellum (see Supplementary Figures 17a-17d). The blood-brain methylation correlation for *BRSK2* CpG5 is not provided since the correlations provided by the Blood Brain DNA Methylation Comparison Tool is based on the Illumina 450K array and CpG5 was not included on this array. There were no significant correlations between blood and brain methylation for the *ADCYAP1* CpG sites (see Supplementary Figures 18a and 18b).

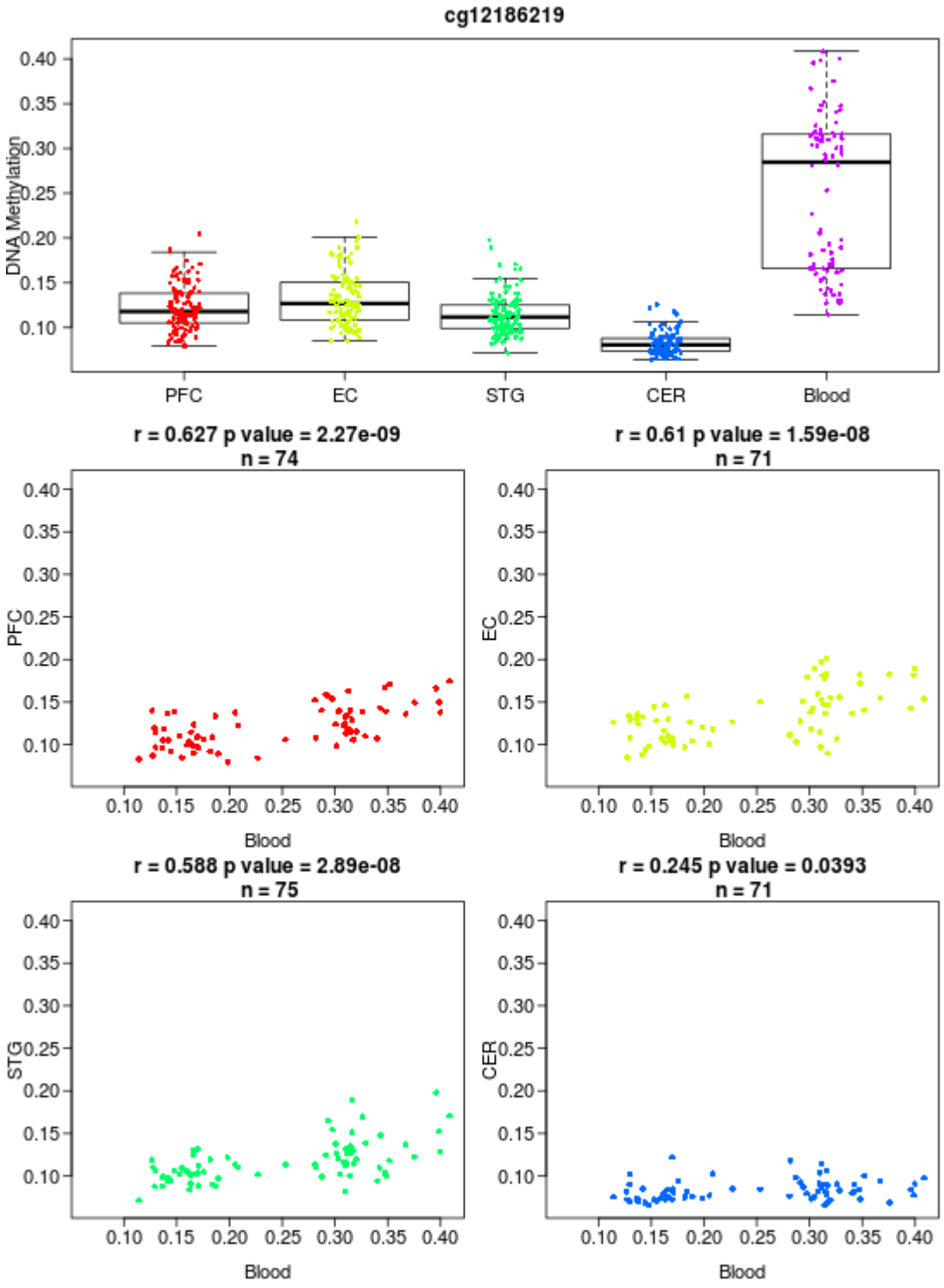


Figure 16a: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *BRSK2* CpG1 (cg12186219).

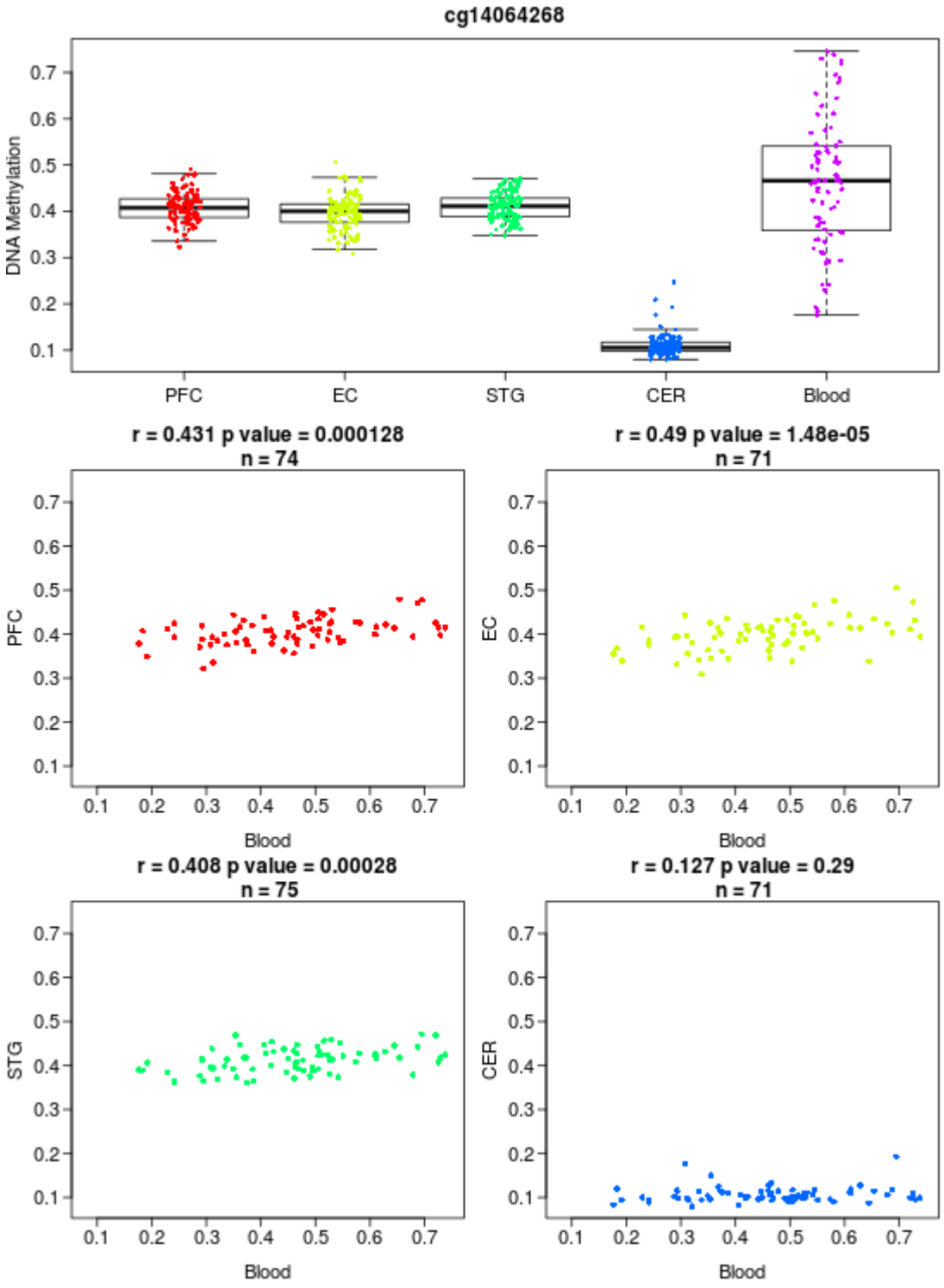


Figure 16b: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *BRSK2* CpG2 (cg14064268).

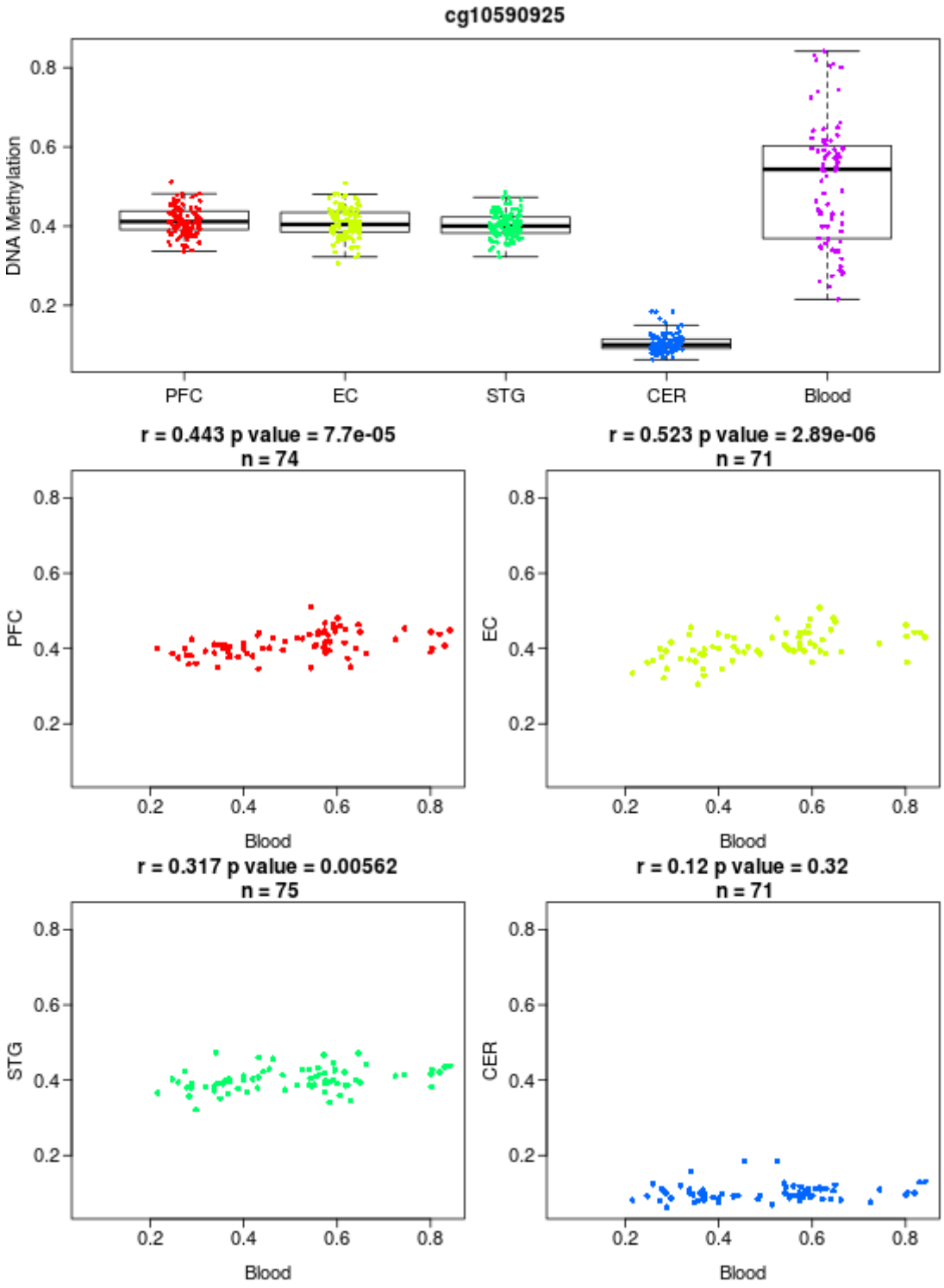


Figure 16c: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *BRSK2* CpG3 (cg10590925).

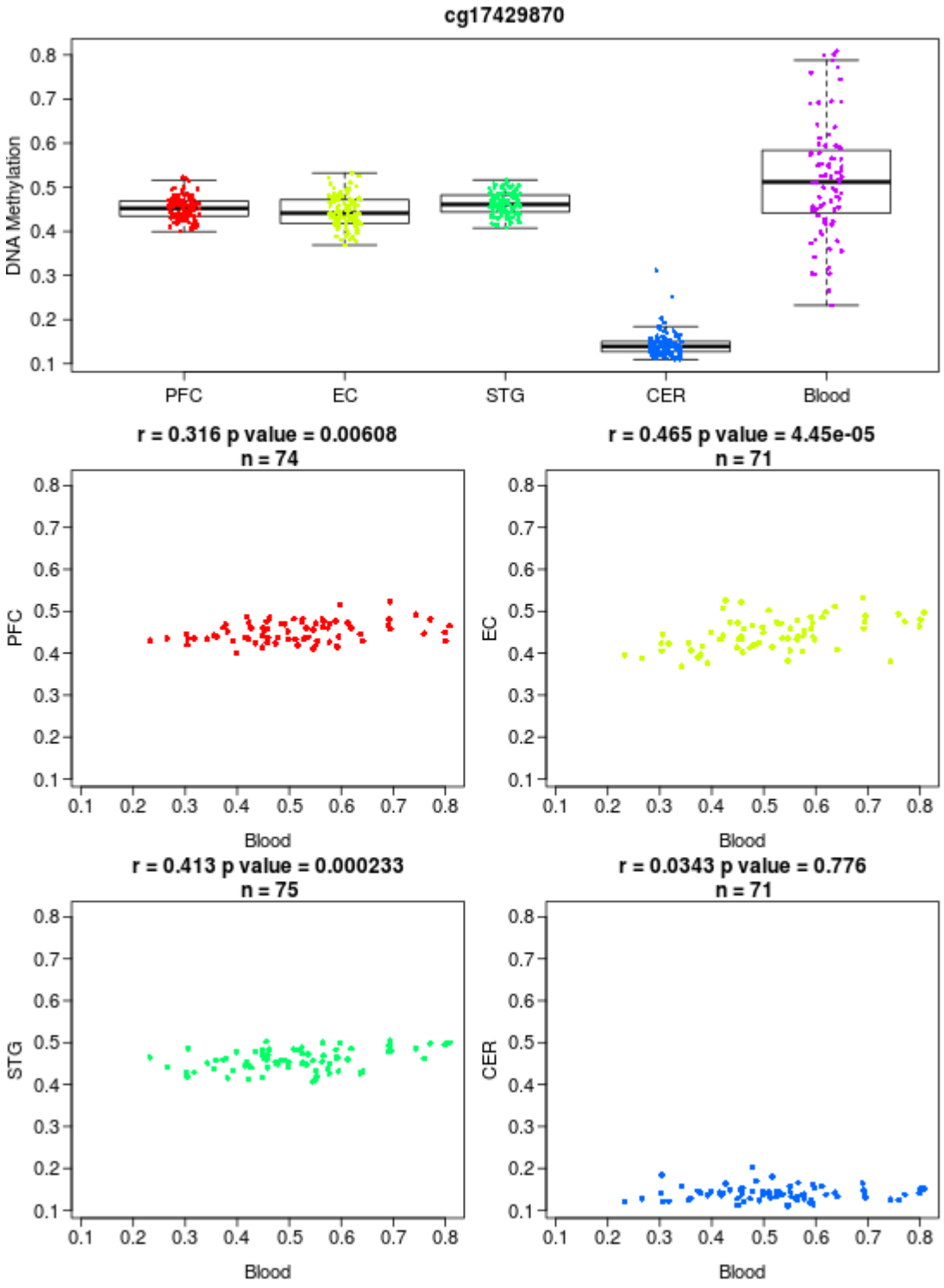


Figure 16d: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *BRSK2* CpG4 (cg17429870).

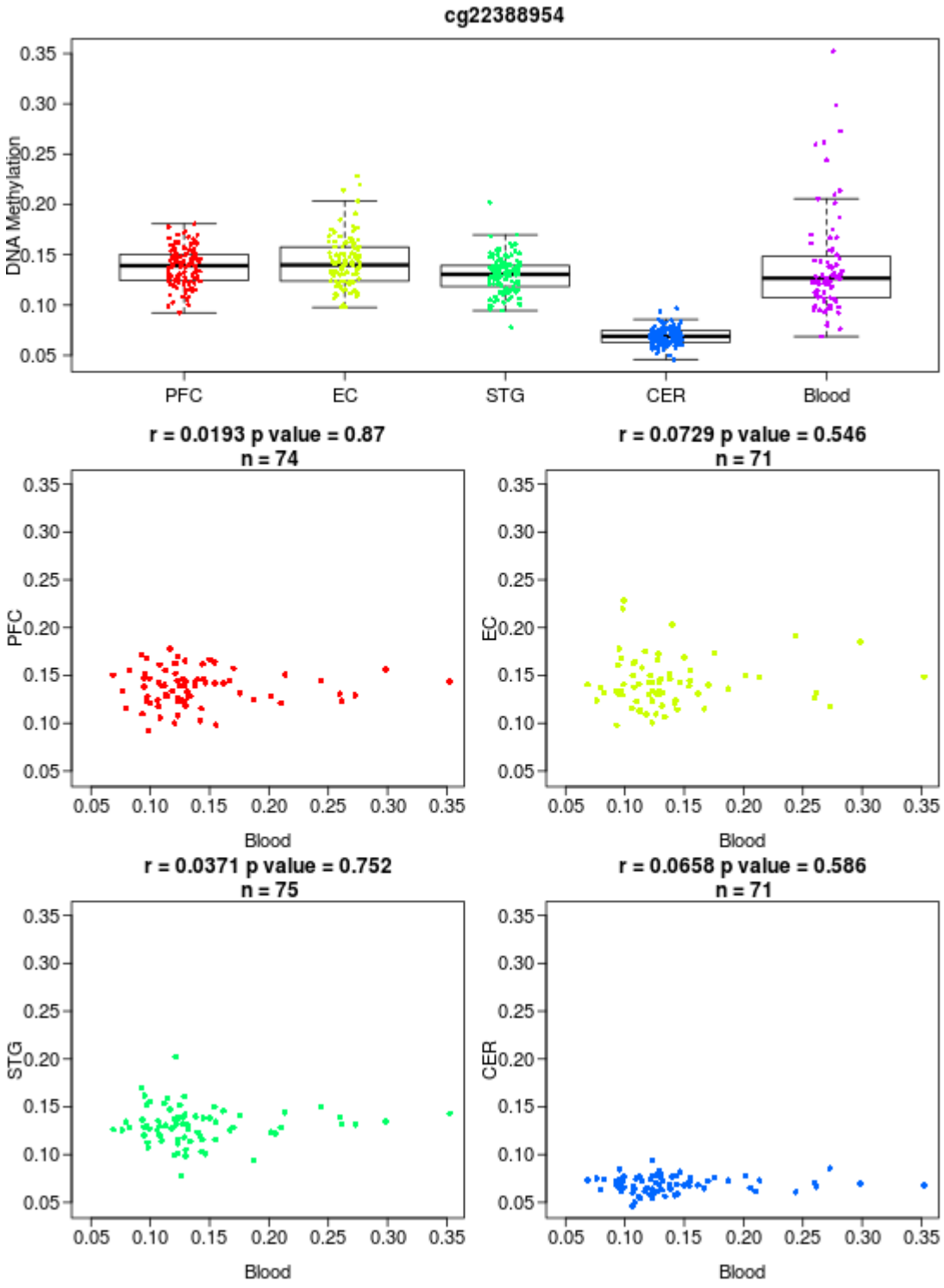


Figure 17a: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *ADCYAP1* CpG5 (cg22388954).

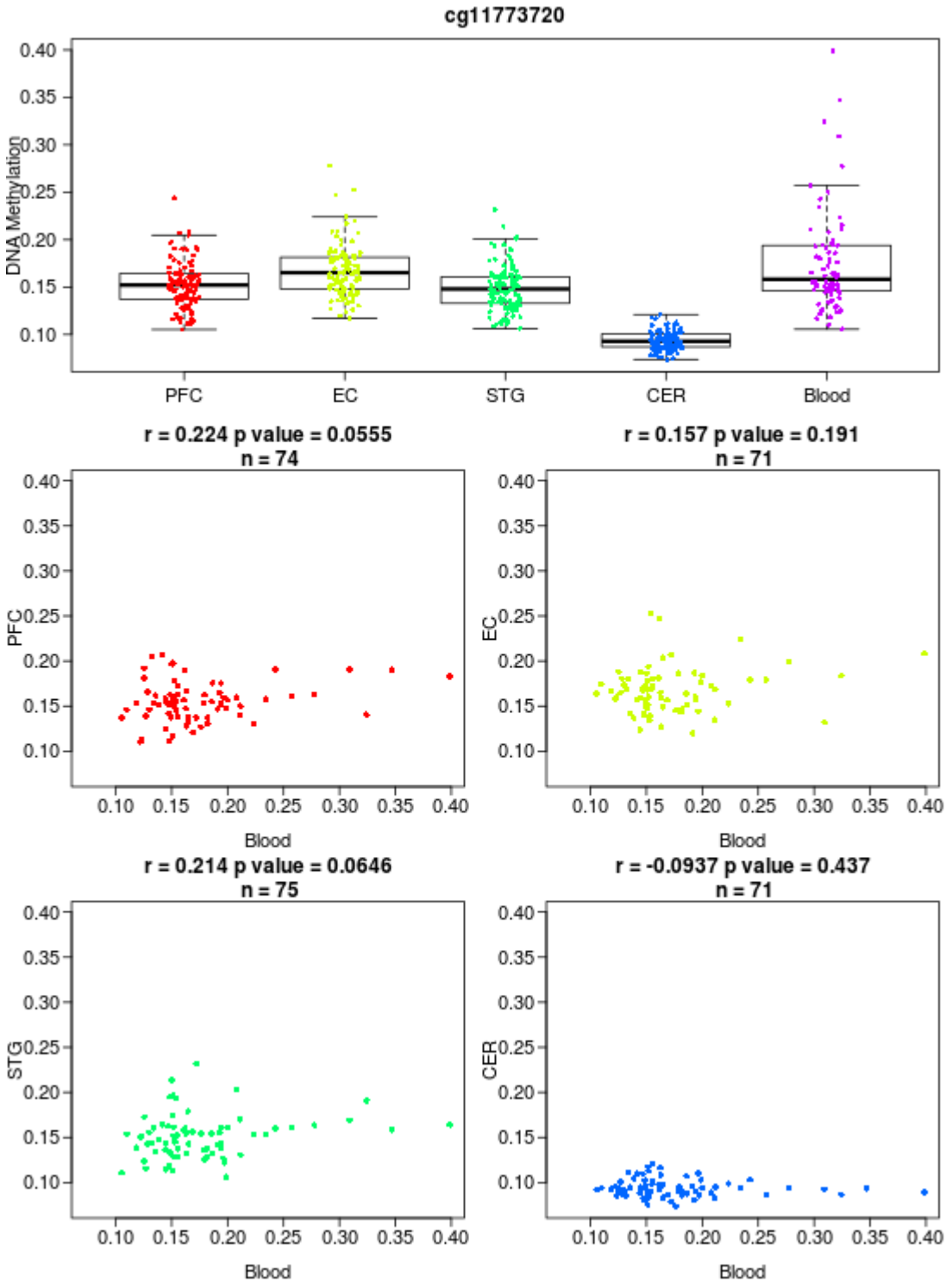


Figure 17b: Correlation between methylation levels observed in blood and methylation levels observed in the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG) and the cerebellum (CER) for *ADCYAP1* CpG5 (cg11773720).

Supplementary Table 4: *Genomic coordinates of the BRSK2 CpG sites investigated longitudinally*

Coordinates	Sequence
chr11:1463561	TGATTGGCTGCCTATGACATCACCAGGCTGGGCTGCTATTGG
chr11:1463603	CCCTTATGTGTGATTGG**CG**TTTGGAGAGGCAGTGGGCTCTG
chr11:1463644	GGCAGGGGGTCTCCAGGG**CG**GGGAGG**CG**CTCAAGGCAGA
chr11:1463683	GACTGGCCCTGTTCAGCCTCACCACCCTCCTCCCCAGCCACA
chr11:1463725	GGGATCTGAAACCTGAAAACCTCCTGCTGGACGAGAAGAAC

CpG3 CpG4 CpG5

Genomic coordinates are based on the Human Genome Build 37 (GRCh37/hg19).

Supplementary Table 5: *Univariate relationship between baseline confounding/covarying factors, PTSD, BRSK2 and ADCYAP1 methylation in the discovery/validation set*

	Age baseline		HIV status (negative vs positive)		BMI baseline		Smoker (no vs yes)		Childhood trauma baseline		Lifetime trauma baseline		Alcohol use baseline		Depression baseline		Medication use (no vs yes)	
	r/z	*p*	z/x^2	*p*	*r/z*	*p*	z/x^2	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	z/x^2	*p*
PTSD status (3-months)	-1.05	.296	0.02	.900	-1.11	.268	0.18	.672	-1.66	.098	-1.86	.063	-0.85	.394	-1.36	.173	0.17	.680
BRSK2 CpG3 (3-months)	0.02	.881	-0.44	.657	-0.04	.810	-0.07	.944	-0.16	.282	-0.16	.301	0.02	.889	0.17	.259	-0.56	.573
BRSK2 CpG4 (3-months)	-0.07	.650	-0.17	.868	-0.10	.516	-0.23	.818	-0.14	.354	-0.19	.210	0.12	.443	0.17	.263	-0.82	.935
BRSK2 CpG5 (3-months)	0.01	.946	-0.34	.731	-0.10	.516	-0.12	.902	-0.10	.527	-0.12	.429	0.10	.505	0.19	.208	-0.52	.606
ADCYAP1 CpG5.6 (3-months)	-0.15	.341	-1.47	.141	-0.13	.400	-1.32	.188	0.17	.273	0.07	.648	0.26	.083	0.10	.512	-0.22	.823

Abbreviations:
Posttraumatic stress disorder (PTSD), brain-specific serine/threonine-protein kinase 2 (*BRSK2*), adenylate cyclase activating polypeptide 1 (*ADCYAP1)*

Supplementary Table 6: *Univariate relationship between baseline confounding/covarying factors, PTSD, BRSK2 and ADCYAP1 methylation in the replication set*

	Age baseline		HIV status (negative vs positive)		BMI baseline		Smoker (no vs yes)		Childhood trauma baseline		Lifetime trauma baseline		Alcohol use baseline		Depression baseline		Medication use (no vs yes)	
	r/z	*p*	*z/x²*	*p*	*r/z*	*p*	*z/x²*	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	*z/x²*	*p*
PTSD status (3-months)	-0.50	.616	0.57	.451	-0.33	.745	0.17	.899	-1.04	.299	-2.47	.014*	-1.04	.299	-1.45	.149	0.62	.433
BRSK2 CpG3 (3-months)	-0.04	.769	-0.61	.545	-0.05	.725	-0.14	.989	-0.22	.133	-0.14	.329	0.15	.302	0.08	.587	-0.96	.339
BRSK2 CpG4 (3-months)	-0.06	.702	-1.11	.267	0.13	.360	-0.82	.415	0.14	.324	-0.14	.349	0.10	.497	0.17	.240	-1.42	.156
BRSK2 CpG5 (3-months)	-0.08	.571	-1.23	.220	0.05	.726	-0.67	.501	-0.10	.495	-0.15	.305	0.03	.836	0.08	.579	-1.67	.094
ADCYAP1 CpG5.6 (3-months)	0.04	.785	-1.85	.064	0.09	.557	-0.54	.586	0.09	.551	0.08	.576	-0.24	.095	0.04	.796	-1.43	.154

Abbreviations:
Posttraumatic stress disorder (PTSD), brain-specific serine/threonine-protein kinase 2 (*BRSK2*), adenylate cyclase activating polypeptide 1 (*ADCYAP1)*

Supplementary Table 7: *Validation of the BRSK2 and ADCYAP1 findings resulting from the epigenome-wide association study and the discovery set*

Model		β	SE	Wald's χ^2	*p*	OR	Lower	Upper
	***BRSK2* CpG3 methylation**							
1A	CpG3 (3-months)	-0.04	0.02	3.84	.050	0.96	0.92	1.00
1B	CpG3 (3-months)	-0.04	0.02	3.20	.074	0.96	0.92	1.00
	Childhood trauma (baseline)	0.11	0.09	1.54	.215	1.11	0.94	1.31
	***BRSK2* CpG4 methylation**							
2A	CpG4 (3-months)	-0.04	0.02	3.78	.052	0.96	0.92	1.00
2B	CpG4 (3-months)	-0.04	0.02	3.25	.072	0.96	0.92	1.00
	Childhood trauma (baseline)	0.11	0.08	1.66	.198	1.11	0.95	1.31
	***BRSK2* CpG5 methylation**							
3A	CpG5 (3-months)	-0.04	0.02	3.90	.048	0.96	.928	1.00
3B	CpG5 (3-months)	-0.04	0.02	3.32	.069	0.97	0.93	1.00
	Childhood trauma (baseline)	0.11	0.09	1.58	0.21	1.11	0.94	1.32
	***ADCYAP1* CpG1&2 methylation**							
4A	*ADCYAP1* CpG5.6 (3-months)	-0.09	0.10	0.76	.382	0.92	0.76	1.11
4B	*ADCYAP1* CpG5.6 (3-months)	-0.13	0.11	1.25	.263	0.88	0.71	1.10
	Childhood trauma (baseline)	0.14	0.08	2.66	.103	1.15	0.97	1.35

Abbreviations: standard error (SE); odds ration (OR), brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; adenylate cyclase activating polypeptide 1 *(ADCYAP1)*.

Supplementary Table 8: *Replication of the BRSK2 and ADCYAP1 findings resulting from the epigenome-wide association study and the discovery set*

Model		β	SE	Wald's χ^2	*p*	OR	Lower	Upper
	***BRSK2* CpG3 methylation**							
1A	CpG3 (3-months)	-0.00	0.02	0.02	.889	1.00	0.96	1.04
1B	CpG3 (3-months)	-0.01	0.02	0.11	.736	0.99	0.95	1.04
	Childhood trauma (baseline)	-0.12	0.14	0.84	.360	0.88	0.68	1.15
	***BRSK2* CpG4 methylation**							
2A	CpG4 (3-months)	-0.01	0.02	0.19	.667	0.99	0.95	1.04
2B	CpG4 (3-months)	-0.01	0.02	0.32	.570	0.99	0.94	1.03
	Childhood trauma (baseline)	-0.13	0.14	0.87	.350	0.88	0.68	1.15
	***BRSK2* CpG5 methylation**							
3A	CpG5 (3-months)	0.00	0.02	0.03	.866	1.00	0.97	1.04
3B	CpG5 (3-months)	0.00	0.02	0.01	.944	1.00	0.96	1.04
	Childhood trauma (baseline)	-0.13	0.14	0.88	.347	0.88	0.68	1.15
	***ADCYAP1* CpG1&2 methylation**							
4A	*ADCYAP1* CpG5.6 (3-months)	-0.06	0.13	0.22	.639	0.94	0.73	1.21
4B	*ADCYAP1* CpG5.6 (3-months)	-0.5	0.13	0.15	.700	0.95	0.74	1.22
	Childhood trauma (baseline)	-0.12	0.13	0.83	.362	0.89	0.68	1.15

Abbreviations: standard error (SE); odds ration (OR), brain-specific serine/threonine-protein kinase 2 *(BRSK2)*; adenylate cyclase activating polypeptide 1 *(ADCYAP1)*.

Supplementary Table 9: Genomic coordinates of the *ADCYAP1* CpG sites investigated longitudinally

Coordinates	Sequence
chr18:905159	GTCTGGCTAGTTATTGGG**CGCCG**GGTAGATGCATATATAT
	CpG1 CpG2
chr18:905199	ATATTTTTTTCTAACTATAGCAAGCAAGAAGTGGCAGGG

Genomic coordinates are based on the Human Genome Build 37 (GRCh37/hg19).

Supplementary Table 10*: Comparison of BRSK2 methylation levels obtained from the MethylationEPIC array and the EpiTYPER analysis*

	BRSK2 CpG3 EpiTYPER	*BRSK2* CpG4 EpiTYPER	*BRSK2* CpG5 EpiTYPER	*BRSK2* CpG3 EWAS	*BRSK2* CpG4 EWAS	*BRSK2* CpG5 EWAS
BRSK2 CpG3 EpiTYPER	1.000					
BRSK2 CpG4 EpiTYPER	.899**	1.000				
BRSK2 CpG5 EpiTYPER	.908**	.901**	1.000			
BRSK2 CpG3 EPIC array	.881**	.912**	.859**	1.000		
BRSK2 CpG4 EPIC array	.873**	.900**	.843**	.950**	1.000	
BRSK2 CpG5 EPIC array	.861**	.889**	.831**	.956**	.967**	1.000

*p < .05, **p < .01

Supplementary Table 11: *Comparison of ADCYAP1 methylation levels obtained from the MethylationEPIC array and the EpiTYPER analysis*

	ADCYAP1 CpG1&2 EpiTYPER	*ADCYAP1* CpG1&2 EWAS
ADCYAP1 CpG1&2 EpiTYPER	1.000	
ADCYAP1 CpG1&2 EPIC array	.254	1.000

*p < .05, **p < .01

Supplementary Table 12: *Summary of genes and CpG sites investigated in candidate gene studies alongside findings in the current epigenome-wide study*

Gene name[1]	Reference	Genomic position[2]	Location in gene[3,4]	CpG Island[3]	Pro-moter[5]	Main finding	*p*-value	Study specific identifier
ADCYAP1								
	Current EWAS	chr18:905101	TSS200; TSS1500; 5'UTR	Island	NSF	↑Methylation in group with PTSD	*p*=.034	cg17059658
	Current EWAS	chr18:905127	TSS200; TSS1500; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.006	cg15194943
	Current EWAS	chr18:905177	TSS200; TSS1500; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.00008	cg22388954
	Current EWAS	chr18:905180	TSS200; TSS1500; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.0008	cg11773720
	Current EWAS	chr18:905245	TSS200; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.044	cg09172003
	Ressler et al., 2011[57]	chr18:905450	NSF	NSF	NSF	Not significant	*p*>.05	cg07376535
ADCYAP1R1								
	Ressler et al., 2011[57]	chr7:31091718	TSS1500	Island	NSF	↑ Methylation ↑ PTSD symptom severity	*p*<.0005*	cg27076139
	Current EWAS	chr7:31092854	5'UTR	Island	NSF	↓ Methylation in group with PTSD	*p*=.012	cg11218385
AIM2								
	Miller et al., 2018[58]	chr1:159046973	TSS1500	NSF	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.009*	cg10636246
BDNF								
	Current EWAS	chr11:27681475	TSS1500; 3'UTR; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.017	cg07238832
	Current EWAS	chr11:27722889	TSS1500; 3'UTR; 5'UTR	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.029	cg04672351
	Current EWAS	chr11:27732958	3'UTR; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.029	cg11806762
	Moser et al., 2015[59]	chr11:27744022[6]	Exon IV	NSF	Yes	Not significant	*p*>.05	CpG4
	Current EWAS	chr11:27744049	TSS1500; 3'UTR	Island	NSF	↓ Methylation in group with PTSD	*p*=.021	cg15462887
	Kim et al., 2017[60]	chr11:27744279[6]	Exon I	NSF	Yes	↑ Methylation in group with PTSD	*p*=.009*	CpG4
	Kim et al., 2017[60]	chr11:27744286[6]	Exon I	NSF	Yes	↑ Methylation in group with PTSD	*p*=.021*	CpG3
	Kim et al., 2017[60]	chr11:27744290[6]	Exon I	NSF	Yes	↑ Methylation in group with PTSD	*p*=.039*	CpG2
	Kim et al., 2017[60]	chr11:27744292[6]	Exon I	NSF	Yes	↑ Methylation in group with PTSD	*p*=.053	CpG1

	Moser et al., 2015[59]	chr11:27744963[6]	Exon IV	NSF	Yes	Not significant	*p*>.05	CpG1
	Moser et al., 2015[59]	chr11:27744971[6]	Exon IV	NSF	Yes	Not significant	*p*>.05	CpG2
	Moser et al., 2015[59]	chr11:27744975[6]	Exon IV	NSF	Yes	Not significant	*p*>.05	CpG3
COMT								
	Norrholm et al., 2013[61]	chr22:19950040	NSF	NSF	Yes	↑ Methylation in group with PTSD	*p*<.01*	cg23601416
	Current EWAS	chr22:19956281	3'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.040	cg19930203
	Current EWAS	chr22:19938096	TSS1500; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.002	cg09926649
	Current EWAS	chr22:19928616	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.015	cg27399558
	Current EWAS	chr22:19928667	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.044	cg11712482
	Current EWAS	chr22:19928740	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.039	cg24547396
	Current EWAS	chr22:19928445	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.0004	cg15926585
	Current EWAS	chr22:19949585	TSS1500; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.028	cg21905167
FKBP5								
		chr6:35558386[6]	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					Not significant	*p*>.05	CpG35558386
	Yehuda et al., 2016[63]					Not significant	*p*>.05	Bin1-CpG1
		chr6:35558438[6]	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					Not significant	*p*>.05	CpG35558438
	Yehuda et al., 2016[63]					Not significant	*p*>.05	Bin1-CpG2
		chr6:35558488	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					Not significant	*p*>.05	CpG35558488
	Yehuda et al, 2016[63]					Not significant	*p*>.05	Bin2-CpG3
	Kang et al., 2019[64]					↑ Methylation in group with PTSD	*p*=.037*	CpG1
		chr6:35558513[6]	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					↑ Methylation in MBSR non-responders post-treatment	*p*=.029*	CpG35558513
	Yehuda et al., 2016[63]					Not significant	*p*>.05	Bin2-CpG4-GRE
	Kang et al., 2019[64]					↑ Methylation in group with PTSD	*p*=.037*	CpG2
		chr6:35558566[6]	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					Not significant	*p*>.05	CpG35558566
	Yehuda et al., 2016[63]					Not significant	*p*>.05	Bin2-CpG5

		chr6:35558710[6]	Intron 7; TSS	NSF	NSF			
	Bishop et al., 2018[62]					Not significant	*p*>.05	CpG35558710
	Yehuda et al., 2016[63]					↑ Methylation in group with PTSD	*p*=.046*	Bin3-CpG6
	Bishop et al., 2018[62]	chr6:35558721[6]	Intron 7; TSS	NSF	NSF	Not significant	*p*>.05	CpG35558721
	Yehuda et al., 2016[63]	chr6:35656916-35656633	exon1	NSF	Yes	↑ Methylation (across 38 CpG sites) ↑ PTSD symptom severity	*p*=.044*	CpG1-CpG38
	Current EWAS	chr6:35681420	5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.046	cg24295963
HTR3A								
	Schechter et al., 2017[65]	chr11:113827917[6]	GRE	NSF	Yes	Not significant	*p*>.05	CpG1_I
	Schechter et al., 2017[65]	chr11:113844529[6]	TSS	NSF	Yes	Not significant	*p*>.05	CpG4_II
	Schechter et al., 2017[65]	chr11:113844828	TSS	NSF	Yes	Not significant	*p*>.05	CpG1_II
	Schechter et al., 2017[65]	chr11:113844864[6]	TSS	NSF	Yes	Not significant	*p*>.05	CpG2_II
	Schechter et al., 2017[65]	chr11:113844942[6]	TSS	NSF	Yes	Not significant	*p*>.05	CpG3_II
	Schechter et al., 2017[65]	chr11:113845939	5'UTR	NSF	Yes	Not significant	*p*>.05	CpG1_III
	Schechter et al., 2017[65]	chr11:113846004	5'UTR	NSF	Yes	↓ Methylation in group with PTSD	*p*=.009*	CpG2_III
	Current EWAS	chr11:113846004	TSS200; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.028	cg20621129
	Schechter et al., 2017[65]	chr11:113846017	5'UTR	NSF	Yes	Not significant	*p*>.05	CpG3_III
	Schechter et al., 2017[65]	chr11:113846044[6]	Coding Sequence	NSF	Yes	↑ Methylation in group with PTSD	*p*=.002*	CpG4_III
	Schechter et al., 2017[65]	chr11:113846070[6]	Coding Sequence	NSF	Yes	↑ Methylation in group with PTSD	*p*=.004*	CpG5_III
	Schechter et al., 2017[65]	chr11:113846077[6]	Coding Sequence	NSF	Yes	Not significant	*p*>.05	CpG6_III
	Current EWAS	chr11:113860607	3'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.036	cg20178075
IL12B								
	Bam et al., 2016[66]	chr5:159314783-159330473	TSS	Island	Yes	↓ Methylation in group with PTSD	NSF	NA
MAN2C1								
	Current EWAS	chr15:75660215	TSS1500; 3'UTR	N_Shore	Yes	↓ Methylation in group with PTSD	*p*=.024	cg05432169
	Uddin et al., 2011[67]	chr15:75661449	NSF	Island	Yes	↑ Methylation x ↑ trauma load in group with PTSD	*p*=.04*	cg04008455
	Current EWAS	chr15:75661532	TSS1500; 5'UTR	S_Shore	Yes	↑ Methylation in group with PTSD	*p*=.029	cg04095413
MAOA								
	Current EWAS	chrX:43515349	TSS200	Island	NSF	↑ Methylation in group with PTSD	*p*=.048	cg19441691
	Ziegler et al., 2018[68]	chrX:43515613[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG1
	Ziegler et al., 2018[68]	chrX:43515624[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG2
	Ziegler et al., 2018[68]	chrX:43515658[6]	Exon1/Intron1	NSF	NSF	↑ Methylation in group with PTSD	*p*=.029*	CpG3

Ziegler et al., 2018[68]	chrX:43515665[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG4
Ziegler et al., 2018[68]	chrX:43515667[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG5
Ziegler et al., 2018[68]	chrX:43515680[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG6
Ziegler et al., 2018[68]	chrX:43515683[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG7
Ziegler et al., 2018[68]	chrX:43515689[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG8
Ziegler et al., 2018[68]	chrX:43515695[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG9
Ziegler et al., 2018[68]	chrX:43515724[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG10
Ziegler et al., 2018[68]	chrX:43515729[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG11
Ziegler et al., 2018[68]	chrX:43515811[6]	Exon1/Intron1	NSF	NSF	↑ Methylation in group with PTSD	*p*=.011*	CpG12
Ziegler et al., 2018[68]	chrX:43515840[6]	Exon1/Intron1	NSF	NSF	Not significant	*p*>.05	CpG13
NR3C1							
Current EWAS	chr5:142729377	3'UTR; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.029	cg22233604
Current EWAS	chr5:142729913	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.031	cg03857453
Current EWAS	chr5:142735238	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.008	cg14621978
Labonte et al., 2014[69]	chr5:142783095[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG54
Labonte et al., 2014[69]	chr5:142783101[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG53
Labonte et al., 2014[69]	chr5:142783104[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG52
Labonte et al., 2014[69]	chr5:142783112[6]	5'UTR, exon 1C	NSF	Yes	↑ Methylation in group with PTSD	*p*<.05*	1C-CpG51
Labonte et al., 2014[69]	chr5:142783120[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG50
Labonte et al., 2014[69]	chr5:142783128[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG49
Labonte et al., 2014[69]	chr5:142783139[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG48
Labonte et al., 2014[69]	chr5:142783145[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG47
Labonte et al., 2014[69]	chr5:142783161[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG46
Labonte et al., 2014[69]	chr5:142783164[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG45
Labonte et al., 2014[69]	chr5:142783167[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG44
Labonte et al., 2014[69]	chr5:142783181[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG43
Labonte et al., 2014[69]	chr5:142783183[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG42
Labonte et al., 2014[69]	chr5:142783189[6]	5'UTR, exon 1C	NSF	Yes	↓ Methylation in group with PTSD	*p*<.05*	1C-CpG41
Labonte et al., 2014[69]	chr5:142783191[6]	5'UTR, exon 1C	NSF	Yes	↓ Methylation in group with PTSD	*p*<.05*	1C-CpG40
Labonte et al., 2014[69]	chr5:142783204[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG39
Labonte et al., 2014[69]	chr5:142783213[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG38
Labonte et al., 2014[69]	chr5:142783217[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG37
Labonte et al., 2014[69]	chr5:142783221[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG36
Labonte et al., 2014[69]	chr5:142783227[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG35
Labonte et al., 2014[69]	chr5:142783231[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG34
Labonte et al., 2014[69]	chr5:142783238[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG33
Labonte et al., 2014[69]	chr5:142783248[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG32
Labonte et al., 2014[69]	chr5:142783251[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG31

Labonte et al., 2014[69]	chr5:142783256[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG30
Labonte et al., 2014[69]	chr5:142783259[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG29
Labonte et al., 2014[69]	chr5:142783261[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG28
Labonte et al., 2014[69]	chr5:142783271[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG27
Labonte et al., 2014[69]	chr5:142783279[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG26
Labonte et al., 2014[69]	chr5:142783281[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG25
Labonte et al., 2014[69]	chr5:142783298[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG24
Labonte et al., 2014[69]	chr5:142783302[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG23
Labonte et al., 2014[69]	chr5:142783309[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG22
Labonte et al., 2014[69]	chr5:142783313[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG21
Labonte et al., 2014[69]	chr5:142783321[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG20
Labonte et al., 2014[69]	chr5:142783323[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG19
Labonte et al., 2014[69]	chr5:142783325[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG18
Labonte et al., 2014[69]	chr5:142783328[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG17
Labonte et al., 2014[69]	chr5:142783332[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG16
Labonte et al., 2014[69]	chr5:142783334[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG15
Labonte et al., 2014[69]	chr5:142783341[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG14
Labonte et al., 2014[69]	chr5:142783360[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG13
Labonte et al., 2014[69]	chr5:142783379	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG12
Labonte et al., 2014[69]	chr5:142783383	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG11
Labonte et al., 2014[69]	chr5:142783385	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG10
Labonte et al., 2014[69]	chr5:142783400[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG9
Labonte et al., 2014[69]	chr5:142783407[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG8
Labonte et al., 2014[69]	chr5:142783409[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG7
Labonte et al., 2014[69]	chr5:142783411[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG6
Labonte et al., 2014[69]	chr5:142783418[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG5
Labonte et al., 2014[69]	chr5:142783426[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG4
Labonte et al., 2014[69]	chr5:142783432[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG3
Labonte et al., 2014[69]	chr5:142783435[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG2
Labonte et al., 2014[69]	chr5:142783438[6]	5'UTR, exon 1C	NSF	Yes	Not significant	*p*>.05	1C-CpG1
	chr5:142783528[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG13
Schur et al., 2017[71]					Not significant	*p*>.05	CpG52
	chr5:142783531[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG12
Schur et al., 2017[71]					Not significant	*p*>.05	CpG51
	chr5:142783537[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG11
Schur et al., 2017[71]					Not significant	*p*>.05	CpG50

	chr5:142783540[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG10
Schur et al., 2017[71]					Not significant	*p*>.05	CpG49
	chr5:142783547[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG9
Schur et al., 2017[71]					Not significant	*p*>.05	CpG48
	chr5:142783566[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG8
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG8
Schur et al., 2017[71]					Not significant	*p*>.05	CpG47
	chr5:142783569	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG7
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG7
Schur et al., 2017[71]					Not significant	*p*>.05	CpG46
	chr5:142783584[6]	5'UTR, exon 1F	Island	Yes			
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG6
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG6
Schur et al., 2017[71]					Not significant	*p*>.05	CpG45
Yehuda et al., 2013[73]	chr5:142783607-142783883	5'UTR, exon 1F	Island	Yes	↑ Methylation (across 39 CpG sites) ↓ PTSD symptom severity	*p*<.05*	CpG1-39
	chr5:142783607	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2013[73]					↓ Methylation in group with PTSD	*p*=.022*	CpG39
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG5
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG26
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG5
Schur et al., 2017[71]					Not significant	*p*>.05	CpG44
	chr5:142783621	5'UTR, exon 1F, NGFI-A binding site	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG38
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG4
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG25
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG4
Schur et al., 2017[71]					Not significant	*p*>.05	CpG43
	chr5:142783627[6]	5'UTR, exon 1F, NGFI-A binding site	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG37

Schechter et al., 2015[70]					Not significant	*p*>.05	CpG3
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG24
Vukojevic et al., 2014[72]					↓ Methylation ↑ PTSD symptom severity	*p*<.05*	CpG3
Schur et al., 2017[71]					Not significant	*p*>.05	CpG42
	chr5:142783637[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG36
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG2
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG23
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG2
Schur et al., 2017[71]					Not significant	*p*>.05	CpG41
	chr5:142783639	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG35
Schechter et al., 2015[70]					Not significant	*p*>.05	CpG1
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG22
Vukojevic et al., 2014[72]					Not significant	*p*>.05	CpG1
Schur et al., 2017[71]					Not significant	*p*>.05	CpG40
	chr5:142783655[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG34
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG21
Schur et al., 2017[71]					Not significant	*p*>.05	CpG39
	chr5:142783663[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG33
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG20
Schur et al., 2017[71]					Not significant	*p*>.05	CpG38
	chr5:142783678[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG32
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG19
Schur et al., 2017[71]					Not significant	*p*>.05	CpG37
	chr5:142783685[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG31
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG18
Schur et al., 2017[71]					Not significant	*p*>.05	CpG36
	chr5:142783688[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG30
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG17
Schur et al., 2017[71]					Not significant	*p*>.05	CpG35
	chr5:142783702[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG29
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG16

Schur et al., 2017[71]					Not significant	*p*>.05	CpG34
	chr5:142783712[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG28
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG15
Schur et al., 2017[71]					Not significant	*p*>.05	CpG33
	chr5:142783716[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG27
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG14
Schur et al., 2017[71]					Not significant	*p*>.05	CpG32
	chr5:142783730[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG26
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG13
Schur et al., 2017[71]					Not significant	*p*>.05	CpG31
	chr5:142783735[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG25
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG12
Schur et al., 2017[71]					Not significant	*p*>.05	CpG30
	chr5:142783742[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG24
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG11
Schur et al., 2017[71]					Not significant	*p*>.05	CpG29
	chr5:142783744[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					↓ Methylation in group with PTSD	*p*=.021*	CpG23
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG10
Schur et al., 2017[71]					Not significant	*p*>.05	CpG28
	chr5:142783755[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG22
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG9
Schur et al., 2017[71]					Not significant	*p*>.05	CpG27
	chr5:142783766[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG21
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG8
Schur et al., 2017[71]					Not significant	*p*>.05	CpG26
	chr5:142783768[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG20
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG7
Schur et al., 2017[71]					Not significant	*p*>.05	CpG25
	chr5:142783771[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG19

Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG6
Schur et al., 2017[71]					Not significant	*p*>.05	CpG24
	chr5:142783774[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG18
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG5
Schur et al., 2017[71]					Not significant	*p*>.05	CpG23
	chr5:142783777[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG17
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG4
Schur et al., 2017[71]					Not significant	*p*>.05	CpG22
	chr5:142783780[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG16
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG3
Schur et al., 2017[71]					Not significant	*p*>.05	CpG21
	chr5:142783785[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG15
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG2
Schur et al., 2017[71]					Not significant	*p*>.05	CpG20
	chr5:142783792[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG14
Mcnerney et al., 2018[74]					Not significant	*p*>.05	CpG1
Schur et al., 2017[71]					Not significant	*p*>.05	CpG19
	chr5:142783809[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG13
Schur et al., 2017[71]					Not significant	*p*>.05	CpG18
	chr5:142783821[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG12
Schur et al., 2017[71]					Not significant	*p*>.05	CpG17
	chr5:142783831[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG11
Schur et al., 2017[71]					Not significant	*p*>.05	CpG16
	chr5:142783837[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG10
Schur et al., 2017[71]					Not significant	*p*>.05	CpG15
	chr5:142783843[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG9
Schur et al., 2017[71]					Not significant	*p*>.05	CpG14
	chr5:142783848[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG8

Schur et al., 2017[71]					Not significant	*p*>.05	CpG13
	chr5:142783853[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG7
Schur et al., 2017[71]					Not significant	*p*>.05	CpG12
	chr5:142783857[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG6
Schur et al., 2017[71]					Not significant	*p*>.05	CpG11
	chr5:142783859[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG5
Schur et al., 2017[71]					Not significant	*p*>.05	CpG10
	chr5:142783863[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG4
Schur et al., 2017[71]					Not significant	*p*>.05	CpG9
	chr5:142783869[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG3
Schur et al., 2017[71]					Not significant	*p*>.05	CpG8
	chr5:142783873[6]	5'UTR, exon 1F	Island	Yes			
					Not significant	*p*>.05	CpG2
Schur et al., 2017[71]					Not significant	*p*>.05	CpG7
	chr5:142783883[6]	5'UTR, exon 1F	Island	Yes			
Yehuda et al., 2015[75]					Not significant	*p*>.05	CpG1
Schur et al., 2017[71]					Not significant	*p*>.05	CpG6
Schur et al., 2017[71]	chr5:142783912[6]	5'UTR, exon 1F	Island	Yes	Not significant	*p*>.05	CpG5
Schur et al., 2017[71]	chr5:142783920[6]	5'UTR, exon 1F	Island	Yes	Not significant	*p*>.05	CpG4
Schur et al., 2017[71]	chr5:142783927[6]	5'UTR, exon 1F	Island	Yes	Not significant	*p*>.05	CpG3
Schur et al., 2017[71]	chr5:142783930[6]	5'UTR, exon 1F	Island	Yes	Not significant	*p*>.05	CpG2
Schur et al., 2017[71]	chr5:142783936[6]	5'UTR, exon 1F	Island	Yes	Not significant	*p*>.05	CpG1
Labonte et al., 2014[69]	chr5:142784071[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG29
Labonte et al., 2014[69]	chr5:142784074[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG28
Labonte et al., 2014[69]	chr5:142784078[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG27
Labonte et al., 2014[69]	chr5:142784082[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG26
Labonte et al., 2014[69]	chr5:142784102[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG25
Labonte et al., 2014[69]	chr5:142784110[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG24
Labonte et al., 2014[69]	chr5:142784115[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG23
Labonte et al., 2014[69]	chr5:142784118[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG22
Labonte et al., 2014[69]	chr5:142784121[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG21
Labonte et al., 2014[69]	chr5:142784124[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG20
Labonte et al., 2014[69]	chr5:142784136[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG19
Labonte et al., 2014[69]	chr5:142784139[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG18

	Labonte et al., 2014[69]	chr5:142784168	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG17
	Labonte et al., 2014[69]	chr5:142784187	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG16
	Labonte et al., 2014[69]	chr5:142784201[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG15
	Labonte et al., 2014[69]	chr5:142784222	5'UTR, exon 1B	NSF	Yes	↓ Methylation in group with PTSD	*p*=.05*	1B-CpG14
	Labonte et al., 2014[69]	chr5:142784242[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG13
	Labonte et al., 2014[69]	chr5:142784278	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG12
	Labonte et al., 2014[69]	chr5:142784304[6]	5'UTR, exon 1B	NSF	Yes	↓ Methylation in group with PTSD	*p*<.05*	1B-CpG11
	Labonte et al., 2014[69]	chr5:142784323	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG10
	Labonte et al., 2014[69]	chr5:142784369[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG9
	Labonte et al., 2014[69]	chr5:142784380[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG8
	Labonte et al., 2014[69]	chr5:142784382	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG7
	Current EWAS	chr5:142784382	TSS1500; 5'UTR	Island	Yes	↑ Methylation in group with PTSD	*p*=.036	cg18849621
	Labonte et al., 2014[69]	chr5:142784394[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG6
	Labonte et al., 2014[69]	chr5:142784412[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG5
	Labonte et al., 2014[69]	chr5:142784435[6]	5'UTR, exon 1B	NSF	Yes	↓ Methylation in group with PTSD	*p*<.005*	1B-CpG4
	Labonte et al., 2014[69]	chr5:142784445[6]	5'UTR, exon 1B	NSF	Yes	↓ Methylation in group with PTSD	*p*<.005*	1B-CpG3
	Labonte et al., 2014[69]	chr5:142784462[6]	5'UTR, exon 1B	NSF	Yes	↓ Methylation in group with PTSD	*p*<.005*	1B-CpG2
	Labonte et al., 2014[69]	chr5:142784522[6]	5'UTR, exon 1B	NSF	Yes	Not significant	*p*>.05	1B-CpG1
	Current EWAS	chr5:142815463	TSS1500	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.003	cg07589972
OXTR								
	Nawijn et al., 2019[76]	chr3:8809464[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG1
	Nawijn et al., 2019[76]	chr3:8809442[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG2
	Nawijn et al., 2019[76]	chr3:8809437[6]	Exon 3	Island	NSF	↑ Methylation in females with PTSD	*p*=.022*	CpG3
	Nawijn et al., 2019[76]	chr3:8809433[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG4
	Nawijn et al., 2019[76]	chr3:8809428[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG5
	Nawijn et al., 2019[76]	chr3:8809425[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG6
	Nawijn et al., 2019[76]	chr3:8809422[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG7
	Nawijn et al., 2019[76]	chr3:8809417[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG8
	Nawijn et al., 2019[76]	chr3:8809413[6]	Exon 3	Island	NSF	↑ Methylation in females with PTSD	*p*=.015*	CpG9
	Nawijn et al., 2019[76]	chr3:8809399[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG10
	Nawijn et al., 2019[76]	chr3:8809394[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG11
	Nawijn et al., 2019[76]	chr3:8809387[6]	Exon 3	Island	NSF	Not significant	*p*>.05	CpG12
SKA2		chr17:57187728	3'UTR	NSF	NSF			
	Sadeh et al., 2016[77]					↑ Methylation ↑ PTSD symptom severity	*p*=.021*	cg13989295
	Boks et al., 2016[78]					↓ Methylation ↑ PTSD symptom severity	*p*=.00006*	cg13989295
	Current EWAS	chr17:57232722	TSS1500; 5'UTR	S_Shore	Yes	↑ Methylation in group with PTSD	*p*=.012	cg20104110

SLC6A3								
	Current EWAS	chr5:1443460	TSS200; TSS1500; 5'UTR	N_Shore	NSF	↑ Methylation in group with P TSD	*p*=.017	cg04088992
	Current EWAS	chr5:1444395	TSS1500; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.023	cg13723431
	Current EWAS	chr5:1444333	TSS1500; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.033	cg08713711
	Current EWAS	chr5:1445542	1stExon; 5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.035	cg04598517
	Change et al., 2012[79]	chr5:1446443	TSS1500	Island	Yes	↑ Methylation x *SLC6A3* VNTR 9R in group with PTSD	*p*=.008*	cg13202751
SLC6A4								
	Koenen et al., 2011[80]	chr17:28562220	5'UTR; 1stIntron	N Shore	NSF	↓ Methylation in group with PTSD x ↑ lifetime trauma exposure	*p*=.036*	cg22584138

[1] Identified using the GENECODE database; [2] identified using the Human Genome 19 (HG19) build from the Genome Reference Consortium;
[3] identified using the University of California Santa Cruz (UCSC) Genomic Institute/Genome Browser; [4] Multiple listings indicate splice variants;
[5] identified using the Methylation Consortium project; [6] CpG site not included on the MethylationEPIC beadchip.
*$p < .05$ after correction for multiple testing

Abbreviations:
Pituitary adenylate cyclase-activating polypeptide 1 *(ADCYAP1)*; epigenome-wide association study (EWAS); transcription start site 200 (TSS200); transcription start site 1500 (TSS1500); 5' untranslated region (5'UTR); not specified (NSF); posttraumatic stress disorder (PTSD); ADCYAP1 receptor 1 *(ADCYAP1R1)*; interferon-inducible protein / absent in melanoma 2 *(AIM2)*; brain-derived neurotropic factor *(BDNF)*; 3' untranslated region (3'UTR); South shore (S_Shore); catechol-O-methyltransferase *(COMT)*; North shore (N_Shore); FK506 binding protein *(FKBP5)*; transcription start site (TSS); mindfulness based stress reduction (MBSR); glucocorticoid response element (GRE); 5-hydroxytryptamine receptor 3A *(HTR3A)*; interleukin 12B *(IL12B)*; mannosidase, alpha class 2c member 1 *(MAN2C1)*; monoamine oxidase A *(MAOA)*; nuclear receptor subfamily 3, group C *(NR3C1)*; transcription factor nerve growth factor-inducible protein A (NGFI-A); oxytocin receptor *(OXTR)*; spindle and kinetochore-associated protein 2 *(SKA2)*; 3' untranslated region (3'UTR); solute carrier family 6, member 3 *(SLC6A3)*; variable number tandem repeat nine repeats (VNTR 9R); solute carrier family 6, member 4 *(SLC6A4)*.

Supplementary Table 13: *Summary of genes and CpG sites associations with PTSD observed in prior epigenome-wide studies alongside associations observed in the current study*

Gene[1]	Reference	Genomic position[2]	Illumina ID	Location in gene[3,4]	CpG Island[3]	Pro-moter[5]	Finding	*p*-value
ACO11899.9								
	Smith et al., 2019[81]	Chr7:157294357	cg26801037	Intergenic	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000308
ACP5								
	Smith et al., 2011[82]	Chr19:11688246	cg07967308	NSF	NSF	NSF	↑ Methylation in group with PTSD	*p*=.0000080
	Current EWAS	chr19:11685647	cg14188508	3'UTR	N_Shelf	NSF	↓ Methylation in group with PTSD	*p*=.046
	Current EWAS	chr19:11689855	cg03302259	TSS200/1500	S_Shore	Yes	↓ Methylation in group with PTSD	*p*=.049
AHRR								
	Current EWAS	Chr5:306699	cg21813876	TSS200; 3'UTR; 5'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.001
	Current EWAS	Chr5:314553	cg18584368	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.024
	Current EWAS	Chr5:321320	cg11554391	TSS1500; 5'UTR	Island	NSF	↓ Methylation in group with PTSD	*p*=.044
	Current EWAS	Chr5:369088	cg12202185	5'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.039
		Chr5:373378	cg05575921	5'UTR	N_Shore	NSF		
	Smith et al., 2019[81]						↓ Methylation in group with PTSD	*p*=.0000000000472
	Current EWAS						↓ Methylation in group with PTSD	*p*=.033
	Current EWAS	Chr5:374425	cg22356527	5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.015
		Chr5:377358	cg26703534	5'UTR	S_Shelf	NSF		
	Smith et al., 2019[81]						↓ Methylation in group with PTSD	*p*=.0000000225
	Current EWAS						↓ Methylation in group with PTSD	*p*=.031
	Current EWAS	Chr5:378854	cg01097768	5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.048
	Current EWAS	Chr5:388196	cg01958142	5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.030
	Smith et al., 2019[81]	Chr5:395444	cg25648203	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.0000000107
	Smith et al., 2019[81]	Chr5:399360	cg21161138	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000000439
	Current EWAS	Chr5:405488	cg12207033	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.044
	Current EWAS	Chr5:405567	cg01141993	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.007
ANXA2								
	Smith et al., 2011[82]	Chr15:60690219	cg08081036	NSF	NSF	NSF	↓ Methylation in group with PTSD	*p*=.0000093
ATP9A								

	Current EWAS	Chr20:50224119	cg08403379	Body	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.035
	Smith et al., 2019[81]	Chr20:50312490	cg07339236	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000239
BRSK1								
	Current EWAS	Chr19:55798650	cg24351452	Body	Island	NSF	↓ Methylation in group with PTSD	*p*=.004
	Current EWAS	Chr19:55798477	cg22577385	Body	Island	NSF	↓ Methylation in group with PTSD	*p*=.030
	Mehta et al., 2017[83]	Chr19:55813339	cg02357741	Body	N_Shore	Yes	↓ Methylation in group with PTSD	*p*=.00000224
CDH15								
	Snijders et al., 2020[84]	Chr16:89251975	cg05901543	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.0000025
CLEC9A								
	Current EWAS	Chr12:10183167	cg13830870	TSS200; 3'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.038
	Current EWAS	Chr12:10183220	cg26262442	TSS200	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.010
	Current EWAS	Chr12:10183234	cg02930518	TSS200	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.046
	Smith et al., 2011[82]	Chr12:10183364	cg20098659	NSF	NSF	NSF	↑ Methylation in group with PTSD	*p*=.0000043
	Current EWAS	Chr12:10184399	cg03900817	TSS1500; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.035
COL1A2								
	Rutten et al., 2018[85]	Chr7:93740160	cg22676075	NSF	NSF	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.0000000129
	Rutten et al., 2018[85]	Chr7:94023308	cg24406898	TSS1500	N_Shore	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.00000179
	Current EWAS	Chr7:94059900	cg20943251	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.013
CTRC								
	Snijders et al., 2020[84]	Chr1:15764093	cg18917957	TSS1500	NSF	NSF	↓ Methylation in group with PTSD	*p*=.00000050
	Current EWAS	Chr1:15773099	cg13551783	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.023
DOCK2								
	Current EWAS	Chr5:169064897	cg23184477	5'UTR	S_Shore	NSF	↑ Methylation in group with PTSD	*p*=.032
	Mehta et al., 2017[83]	Chr5:169068404	cg16277944	5'UTR	S_shelf	Yes	↓ Methylation in group with PTSD	*p*=.00000495
	Current EWAS	Chr5:169083252	cg14784010	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.015
	Current EWAS	Chr5:169146570	cg26982433	5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.002
	Current EWAS	Chr5:169416192	cg10849016	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.021
DUSP22								
		Chr6:291882	cg21548813	TSS1500	N_Shore	Yes		
	Rutten et al., 2018[85]						↓ Methylation ↑ PTSD symptom severity	*p*=.0000000140
	Current EWAS						↓ Methylation in group with PTSD	*p*=.032
	Rutten et al., 2018[85]	Chr6:291903	cg03395511	TSS200	N_Shore	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.000000234

	Rutten et al., 2018[85]	Chr6:292329	cg18110333	5'UTR	Island	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.00000000689
	Rutten et al., 2018[85]	Chr6:292522	cg11235426	5'UTR	Island	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.000000665
	Rutten et al., 2018[85]	Chr6:292596	cg01516881	Body	Islan	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.000000452
FLJ46321								
	Smith et al., 2019[81]	Chr9:84609982	cg14405344	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000786
G0S2								
	Logue et al., 2020[86]	Chr1:209849006	cg19534438	5'UTR	Island	NSF	↑ Methylation in group with PTSD	*p*=.000000119
HDAC4								
	Current EWAS	chr2:240062051	cg25828346	3'UTR; 5'UTR	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.033
	Current EWAS	chr2:240066248	cg06107260	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.046
	Maddox et al., 2018[87]	chr2:240168862	cg22937172	5'UTR	NSF	NSF	↑ Methylation in group with PTSD	*p*=.000000127
	Current EWAS	chr2:240196171	cg16348668	3'UTR; 5'UTR	N_Shore	Yes	↑ Methylation in group with PTSD	*p*=.017
	Current EWAS	chr2:240205260	cg00116699	3'UTR; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.013
	Current EWAS	chr2:240205602	cg20453985	3'UTR; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.039
	Current EWAS	chr2:240218482	cg09664216	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.022
	Current EWAS	chr2:240288826	cg06381933	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.004
	Current EWAS	chr2:240309587	cg02812817	3'UTR; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.021
	Current EWAS	chr2:240313052	cg00811555	3'UTR; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.008
HEXDC								
	Current EWAS	Chr17:80375674	cg07050946	TSS1500	N_Shore	Yes	↓ Methylation in group with PTSD	*p*=.020
	Current EWAS	Chr17:80376872	cg12655836	TSS1500; 5'UTR	S_Shore	Yes	↓ Methylation in group with PTSD	*p*=.012
	Snijders et al., 2020[84]	Chr17:80394529	cg20756026	Body	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.000000013
HGS								
	Uddin et al., 2018[88]	Chr17:79658554	cg19577098	Body	S_Shelf	NSF	↓ Methylation in group with PTSD	*p*=.000000147
HIST1H2APS2								
	Rutten et al., 2018[85]	chr6:25882590	cg03517284	NSF	S_Shore	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.013
HOOK2								
	Current EWAS	Chr19:12874033	cg07798386	3'UTR	N_Shelf	NSF	↑ Methylation in group with PTSD	*p*=.002
	Rutten et al., 2018[85]	Chr19:12876846	cg06417478	Body	N_Shore	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.000000000288
	Rutten et al., 2018[85]	Chr19:12876947	cg04657146	Body	Island	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.0000000345
	Rutten et al., 2018[85]	Chr19:12877000	cg11738485	Body	Island	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.0000000129

LCN8								
	Current EWAS	Chr9:139652023	cg16004906	TSS200/1500	N_Shelf	NSF	↓ Methylation in group with PTSD	*p*=.016
	Current EWAS	Chr9:139653461	cg13839917	TSS1500	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.031
	Mehta et al., 2017[83]	Chr9:139653533	cg09325682	TSS1500	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.00000328
LINC00599								
	Smith et al., 2019[81]	Chr8:9742024	cg18217048	Intergenic	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000961
LRRC3B								
	Current EWAS	Chr3:26663805	cg10332616	TSS1500; 3'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.021
	Current EWAS	Chr3:26664115	cg13787438	TSS200/1500; 3'UTR	Island	NSF	↓ Methylation in group with PTSD	*p*=.029
	Current EWAS	Chr3:26688128	cg12975201	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.013
	Mehta et al., 2017[83]	Chr3:26753752	cg26499155	NSF	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.000000794
MAD1L1								
	Snijders et al., 2020[84]	Chr7:1923695	cg12169700	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.00000000081
	Current EWAS	Chr7:2020501	cg17633015	TSS200	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.015
	Current EWAS	Chr7:2250274	cg01859191	3'UTR	Island	NSF	↓ Methylation in group with PTSD	*p*=.036
	Current EWAS	Chr7:2256023	cg00420390	TSS1500; 3'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.028
	Current EWAS	Chr7:2257611	cg17718984	3'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.044
	Current EWAS	Chr7:2274072	cg11571856	TSS1500; 3'UTR	S_Shore	NSF	↑ Methylation in group with PTSD	*p*=.015
MIR3179								
	Smith et al., 2019[81]	Chr13:98749760	cg17284326	Intergenic	NSF	NSF	↓ Methylation in group with PTSD	*p*=.0000000893
MYT1L								
		Chr2:1817351	cg10075506	Body	OpenSea	NSF		
	Rutten et al., 2018[85]						↓ Methylation ↑ PTSD symptom severity	*p*=.00000128
	Current EWAS						↑ Methylation in group with PTSD	*p*=.036
NGF								
	Current EWAS	Chr1:115829856	cg12647496	5'UTR	OpenSea		↓ Methylation in group with PTSD	*p*=.020
	Mehta et al., 2017[83]	Chr1:115844232	cg17750109	5'UTR	NSF	NSF	↓ Methylation in group with PTSD	*p*=.00000306
	Current EWAS	Chr1:115877962	cg01804281	5'UTR	N_Shelf		↓ Methylation in group with PTSD	*p*=.011
NINJ2								
	Rutten et al., 2018[85]	Chr12:739980	cg14911689	Body	NSF	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.00000000861
	Rutten et al., 2018[85]	Chr12:740100	cg26654770	Body	NSF	Yes	↓ Methylation ↑ PTSD symptom severity	*p*=.000000610

	Current EWAS	Chr12:751121	cg11552370	TSS200/1500; 3'UTR; 5'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.004
	Current EWAS	Chr12:751317	cg21334546	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.012
	Current EWAS	Chr12:772861	cg00237010	TSS200; 5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.020
NRG1								
	Uddin et al., 2018[88]	Chr8:31996079	cg23637605	5'UTR	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.0000000753
	Current EWAS	Chr8: 32577951	cg10157574	TSS1500; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.009
	Current EWAS	Chr8:32504400	cg05861515	TSS1500; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.014
	Current EWAS	Chr12:772966	cg15884713	TSS200	OpenSea	NSF	↓ Methylation in group with PTSD	*p*=.038
PAX8								
	Current EWAS	Chr2:113991662	cg19081868	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↓ Methylation in group with PTSD	*p*=.026
	Current EWAS	Chr2:113992694	cg23122642	TSS1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.048
		Chr2:113992921	cg11763394	TSS200/1500; 3'UTR; 5'UTR	N_Shore	NSF		
	Rutten et al., 2018[85]						↓ Methylation ↑ PTSD symptom severity	*p*=.00000129
	Current EWAS						↑ Methylation in group with PTSD	*p*=.030
	Current EWAS	Chr2:113992930	cg21550016	TSS200/1500; 3'UTR; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.047
	Current EWAS	Chr2:113994578	cg00422909	TSS200 /1500; 3'UTR; 5'UTR	S_Shore	NSF	↑ Methylation in group with PTSD	*p*=.0009
	Current EWAS	Chr2:113998034	cg17774569	TSS1500; 5'UTR	S_Shelf	NSF	↓ Methylation in group with PTSD	*p*=.028
	Current EWAS	Chr2:114036444	cg05194362	TSS1500; 3'UTR; 5'UTR	S_Shore	NSF	↑ Methylation in group with PTSD	*p*=.036
RNF6								
	Smith et al., 2019[81]	Chr13:26795862	cg25415650	5'UTR	NSF	NSF	↑ Methylation in group with PTSD	*p*=.0000000738
	Current EWAS	Chr13:26796956	cg27472353	TSS200/1500	S_Shore	Yes	↓ Methylation in group with PTSD	*p*=.015
	Current EWAS	Chr13:26797157	cg03033508	TSS1500	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.013
	Current EWAS	Chr13:26797279	cg23140290	TSS1500	S_Shore	NSF	↓ Methylation in group with PTSD	*p*=.032
SDK1								
	Current EWAS	Chr7:3343581	cg11998431	TSS1500; 5'UTR	S_Shore	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.006
	Current EWAS	Chr7:3343830	cg19871079	5'UTR	S_shelf	NSF	↑ Methylation in group with PTSD	*p*=.034
	Current EWAS	Chr7:3470434	cg02716317	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.043

	Current EWAS	Chr7:3478077	cg25451765	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.008
	Current EWAS	Chr7:3920006	cg18620306	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.002
	Current EWAS	Chr7:3920082	cg05812582	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.014
	Current EWAS	Chr7:3920297	cg05896377	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.012
	Current EWAS	Chr7:3920534	cg18022346	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.042
	Current EWAS	Chr7:3989167	cg01923516	TSS1500; 5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.045
	Current EWAS	Chr7:4018931	cg14950237	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.016
	Current EWAS	Chr7:4049819	cg24851600	5'UTR	OpenSea	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.037
	Current EWAS	Chr7:4052042	cg09274366	5'UTR	OpenSea	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.011
	Current EWAS	Chr7:4056929	cg11082847	5'UTR	OpenSea	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.028
	Current EWAS	Chr7:4146971	cg27215475	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.047
	Current EWAS	Chr7:4152976	cg14682731	5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.009
	Current EWAS	Chr7:4167822	cg12801000	TSS1500; 5'UTR	N_Shore	NSF	↑ Methylation in group with PTSD	*p*=.030
	Current EWAS	Chr7:4175403	cg01667575	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.047
	Current EWAS	Chr7:4183976	cg04612959	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.002
	Current EWAS	Chr7:4191467	cg21108029	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.028
	Current EWAS	Chr7:4213731	cg06610094	TSS200; 5'UTR	OpenSea	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.008
	Current EWAS	Chr7:4228775	cg23357832	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.039
	Current EWAS	Chr7:4228948	cg26800883	5'UTR	OpenSea	NSF	↑ Methylation in group with PTSD	*p*=.034
	Rutten et al., 2018[85]	Chr7:4244643	cg07249765	Body	NSF	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.0000000541
	Current EWAS	Chr7:4276193	cg03010092	5'UTR	OpenSea	NSF	↓ Methylation ↑ PTSD symptom severity	*p*=.006
	Snijders et al., 2020[84]	Chr7:4304779	cg16956686	Body	NSF	NSF	↓ Methylation in group with PTSD	*p*=.00000020
	Current EWAS	Chr7:4306321	cg22611217	3'UTR; 5'UTR	S_Shore	NSF	↑ Methylation in group with PTSD	*p*=.001
	Current EWAS	Chr7:4308120	cg21853331	3'UTR; 5'UTR	S_shelf	NSF	↑ Methylation in group with PTSD	*p*=.009
SPRY4								
	Snijders et al., 2020[84]	Chr5:141660565	cg05656210	NSF	NSF	NSF	↓ Methylation in group with PTSD	*p*=.000000010
TLR8								
	Smith et al., 2011[82]	ChrX:12924783	cg07759587	NSF	NSF	NSF	↑ Methylation in group with PTSD	*p*=.000011
TPR								
		Chr1:186344558	cg24577137	TSS200/1500	Island	Yes		
	Smith et al., 2011[82]						↓ Methylation in group with PTSD	*p*=.0000019
	Current EWAS						↓ Methylation in group with PTSD	*p*=.0008

[1] Identified using the GENECODE database; [2] identified using the Human Genome 19 (HG19) build from the Genome Reference Consortium;
[3] identified using the University of California Santa Cruz (UCSC) Genomic Institute/Genome Browser; [4] Multiple listings indicate splice

variants; [5] identified using the Methylation Consortium project.

Abbreviations:
Anaphase promoting complex subunit 5 (*APC5*); not specified (NSF); posttraumatic stress disorder (PTSD); untranslated region (3'UTR); North shelf (N_Shelf); transcription start site 200 (TSS200); transcription start site 1500 (TSS1500); South shore (S_Shore); human aryl hydrocarbon receptor repressor *(AHRR)*; 5' untranslated region (5'UTR); North shore (N_Shore); South shelf (S_Shelf); annexin A2 (*ANXA2*); ATPase phospholipid transporting 9A *(ATP9A)*; brain-specific serine/threonine-protein kinase 1 *(BRSK1);* cadherin 15 *(CDH15)*; C-type lectin domain family 9, member A (*CLEC9A*); collagen type I alpha 2 chain *(COL1A2);* chymotryosin C *(CTRC);* dedicator of cytokinesis 2 *(DOCK2);* dual specificity phosphatase 22 *(DUSP22)*; family with sequence similarity 75, member D1 *(FLJ46321);* putative lymphocyte G0/G1 switch *(G0S2);* histone deacetylase 4 *(HDAC4);* hexosaminidase glycosyl hydrolase family 20 catalytic domain containing *(HEXDC);* hepatocyte growth factor-regulated tyrosine kinase substrate *(HGS)* H2A histone family, member T, pseudogene *(HIST1H2APS2);* hook microtubule tethering protein 2 *(HOOK2)*; lipocalin 8 *(LCN8)*; long intergenic non-protein coding RNA 599 *(LINC00599);* leucine rich repeat containing 3B *(LRRC3B);* mitotic arrest deficient 1 like 1 *(MAD1L1);* microRNA 3170 *(MIR3170)*; myelin transcription factor 1 like *(MYT1L);* nerve growth factor *(NGF)*; ninjurin 2 *(NINJ2)*; neuregulin 1 *(NRG1);* paired box 8 *(PAX8)*; ring finger protein 6 *(RNF6)*; sidekick cell adhesion molecule 1 *(SDK1)*; sprouty RTK signalling antagonist 4 *(SPRY4)*; toll-like receptor 8 (*TLR8*); translocated promoter region (*TPR*).

Supplementary Table 14: *Univariate relationship baseline confounding/covarying factors, PTSD, BRSK2 and ADCYAP1 methylation*

	Age baseline		HIV status (negative vs positive)		BMI baseline		Smoker (no vs yes)		Childhood trauma baseline		Lifetime trauma baseline		Alcohol use baseline		Depression baseline		Medication use (no vs yes)	
	r/z	*p*	*z/x²*	*p*	*r/z*	*p*	*z/ x²*	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	*r/z*	*p*	*z/x²*	*p*
PTSD total score (baseline)	-0.20	.052	-1.07	.284	0.08	.455	-.487	.626	0.25	.013*	0.06	.550	-0.07	.514	0.50	.000*	-1.46	.144
PTSD total score (3-months)	-0.12	.252	-.139	.889	-0.04	.720	.000	1.00	0.16	.118	0.06	.552	-0.05	.650	0.06	.568*	-1.08	.282
PTSD status (3-months)	0.12	.908	0.93	.336	0.99	.323	0.01	.937	0.70	.482	0.14	.889	0.38	.706	0.14	.890	0.78	.378
PTSD total score (6-months)	0.11	.286	-.631	.528	-0.02	.884	-.288	.773	0.04	.723	0.05	.646	-0.20	.049*	0.22	.029*	-.513	.608
PTSD status (6-months)	1.93	.054	0.50	.478	0.51	.609	0.27	.602	0.86	.389	1.80	.071	1.41	.158	2.01	.044*	0.42	.516
BRSK2 CpG3 (baseline)	-0.01	.918	-1.01	.313	-0.05	.662	-.316	.752	-0.19	.070	-0.19	.061	0.10	.332	0.11	.287	-.540	.589
BRSK2 CpG4 (baseline)	-0.04	.725	-1.01	.313	-0.09	.382	-.543	.587	-0.26	.011*	-0.31	.003*	0.09	.405	0.11	.269	-.933	.351
BRSK2 CpG5 (baseline)	-0.10	.335	-1.38	.169	-0.08	.466	-.587	.557	-0.22	.032*	-0.27	.008*	0.08	.457	0.16	123	-1.57	.117
BRSK2 CpG3 (3-months)	-0.01	.905	-.332	.740	0.02	.844	-.062	.951	-0.16	.114	-0.14	.169	0.08	.466	0.13	.208	-.307	.759
BRSK2 CpG4 (3-months)	-0.07	.508	-1.15	.249	0.08	.451	-.471	.638	-0.15	.149	-0.21	.040*	0.11	.292	0.18	.086	-1.05	.295
BRSK2 CpG5 (3-months)	-0.06	.581	-.853	.394	0.04	.740	-.419	.675	-.127	.224	-0.13	.214	0.06	.560	0.14	.190	-.853	.394
BRSK2 CpG3 (6-months)	-0.02	.866	-.607	.544	-0.00	.990	-.291	.771	-0.15	.141	-0.26	.012*	0.09	.385	0.20	.058	-.272	.786
BRSK2 CpG4 (6-months)	0.00	.967	-.898	.369	0.02	.860	-.768	.443	-0.10	.335	-0.22	.034*	0.08	.434	0.24	.020*	-.232	.816
BRSK2 CpG5 (6-months)	-0.02	.840	-.935	.350	-0.04	.723	-.224	.823	-0.19	.063	-0.24	.020*	0.08	.445	0.19	.073	-.745	.456
ADCYAP1 CpG1&2 (baseline)	-0.40	.698	-1.43	.154	0.02	.834	-1.34	.179	0.04	.670	-0.07	.513	0.14	.163	0.19	.063	-.548	.583
ADCYAP1 CpG1&2 (3-months)	-0.15	.314	-2.13	.034*	0.11	.488	-1.22	.221	0.28	.066	0.09	.559	0.00	.967	0.12	.269	-.703	.482
ADCYAP1 CpG1&2 (6-months)	-0.14	.162	-.060	.952	-0.11	.306	-1.22	.223	0.02	.815	0.04	.698	0.15	.136	-0.10	.314	-.380	.704

Abbreviations: posttraumatic stress disorder (PTSD), brain-specific serine/threonine-protein kinase 2 (*BRSK2*), adenylate cyclase activating polypeptide 1 (*ADCYAP1*

REFERENCES

1. Abrahams N, Seedat S, Lombard C, et al. Study protocol for a longitudinal study evaluating the impact of rape on women's health and their use of health services in South Africa. *BMJ Open*. 2017;7(9):1-13. doi:10.1136/bmjopen-2017-017296

2. Sheehan D, Yves L, Sheehan K, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59(20):34-57.

3. Lecrubier Y, Sheehan D, Weiler E, et al. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. *Eur Psychiatry*. 1997;12(5):224-231.

4. Marques J, Zuardi A. Validity and applicability of the Mini International Neuropsychiatric Interview administered by family medicine residents in primary health care in Brazil. *Gen Hosp Psychiatry*. 2008;30(4):303-310.

5. Davidson JRT, Book SW, Colket JT, et al. Assessment of a new self-rating scale for post-traumatic stress disorder. *Psychol Med*. 1997;27(1):153-160. doi:10.1017/S0033291796004229

6. McDonald S, Beckham J, Morey R, Calhoun P. The validity and diagnostic efficiency of the Davidson Trauma Scale in military veterans who have served since September 11th 2001. *J Anxiety Disord*. 2009;23(2):247-255. doi:10.1038/jid.2014.371

7. Chirwa ED, Sikweyiya Y, Addo-Lartey AA, et al. Prevalence and risk factors of physical or sexual intimate violence perpetration amongst men in four districts in the central region of Ghana: Baseline findings from a cluster randomised controlled trial. *PLoS One*. 2018;13(3):1-18. doi:10.1371/journal.pone.0191663

8. Bernstein D, Fink L. *Childhood Trauma Questionnaire: A Retrospective Self-Report Manual*. San Antonio: The Psychological Corporation; 1998.

9. Villano C, Cleland C, Rosenblum A, et al. Psychometric utility of the childhood trauma questionnaire with female street-based sex workers. *J Trauma Dissociation*. 2004;5(3):33-41. doi:10.1300/J229v05n03

10. Scher CD, Stein MB, Asmundson GJG, Mccreary DR, Forde DR. The Childhood

Trauma Questionnaire in a community sample: Psychometric properties and normative data. *J Trauma Stress*. 2001;14(4):843-857. doi:10.1023/A:1013058625719

11. Jewkes R, Nduna M, Levin J, et al. A cluster randomized-controlled trial to determine the effectiveness of Stepping Stones in preventing HIV infections and promoting safer sexual behaviour amongst youth in the rural Eastern Cape, South Africa: Trial design, methods and baseline findings. *Trop Med Int Heal*. 2006;11(1):3-16. doi:10.1111/j.1365-3156.2005.01530.x

12. Weathers F, Blake D, Schnurr P, Kaloupek D, Marx B, Keane T. *The Life Events Checklist for DSM-5 (LEC-5)*. USA: National Center for PTSD; 2013.

13. Saunders J, Aasland O, Babor T, De La Fuente J, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction*. 1993;88(6):791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

14. Bush K, Kivlahan D, McDonnel M, Fihn S, Bradley K. The AUDIT Alcohol Consumption Questions (AUDIT-C). *Arch Intern Med*. 1998;158(8):1789-1795. doi:10.1097/00000374-199811000-00034

15. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. *J Stud Alcohol*. 1995;56(4):423-432. doi:10.15288/jsa.1995.56.423

16. Cherpitel CJ, Clark WB. Ethnic Differences in Performance of Screening Instruments for Identifying Harmful Drinking and Alcohol Dependence in the Emergency Room. *Alcohol Clin Exp Res*. 1995;19(3):628-634. doi:10.1111/j.1530-0277.1995.tb01559.x

17. Medina-Mora E, Carreño S, De la Fuente JR. Experience with the Alcohol Use Disorders Identification Test (AUDIT) in Mexico. In: *Recent Developments in Alcoholism*. Boston, MA: Springer US; 1998:383-396. doi:10.1007/0-306-47148-5_19

18. Leung SF, Arthur D. The alcohol use disorders identification test (AUDIT): validation of an instrument for enhancing nursing practice in Hong Kong. *Int J Nurs Stud*. 2000;37(1):57-64. doi:10.1016/S0020-7489(99)00052-8

19. Chinyadza E, Moyo IM, Katsumbe TM, et al. Alcohol problems among patients attending five primary health care clinics in Harare city. *Cent Afr J Med*.

1993;39(2):26-32. http://www.ncbi.nlm.nih.gov/pubmed/8261500.

20. Seale JP. Prevalence of Problem Drinking in a Venezuelan Native American Population. *Alcohol Alcohol*. 2002;37(2):198-204. doi:10.1093/alcalc/37.2.198

21. Simbayi L, Kalichman S, Jooste S, Mathiti V, Cain D, Cherry C. Alcohol use and sexual risks for HIV infection among men and women receiving sexually transmitted infection clinic services in Cape Town, South Africa. *J Stud Alcohol*. 2004;65:434-442.

22. Radloff L. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas*. 1977;1(3):385-401. doi:10.1002/ardp.19552881111

23. Boyd JH. Screening for Depression in a Community Sample. *Arch Gen Psychiatry*. 1982;39(10):1195. doi:10.1001/archpsyc.1982.04290100059010

24. Eller LS, Mahat G. Psychological factors in Nepali former commercial sex workers with HIV. *J Nurs Scholarsh*. 2003;35(1):53-60. doi:10.1111/j.1547-5069.2003.00053.x

25. Ghubash R, Daradkeh TK, Al Naseri KS, Al Bloushi NBA, Al Daheri AM. The performance of the center for epidemiologic study depression scale (CES-D) in an Arab female community. *Int J Soc Psychiatry*. 2000;46(4):241-249. doi:10.1177/002076400004600402

26. Guarnaccia PJ, Angel R, Worobey JL. The factor structure of the CES-D in the Hispanic health and nutrition examination survey: The influences of ethnicity, gender and language. *Soc Sci Med*. 1989;29(1):85-94. doi:10.1016/0277-9536(89)90131-7

27. Iwata N, Buka S. Race/ethnicity and depressive symptoms: a cross-cultural/ethnic comparison among university students in East Asia, North and South America. *Soc Sci Med*. 2002;55(12):2243-2252. doi:10.1016/S0277-9536(02)00003-5

28. Munet-Vilaró F, Folkman S, Gregorich S. Depressive Symptomatology in Three Latino Groups. *West J Nurs Res*. 1999;21(2):209-224. doi:10.1177/01939459922043848

29. Myers J, Weissman M. Use of a self-report symptom scale to detect depression in a community sample. *Am J Psychiatry*. 1980;137(9):1081-1084.

doi:10.1176/ajp.137.9.1081

30. Roberts RE, Rhoades HM, Vernon SW. Using the CES-D scale to screen for depression and anxiety: Effects of language and ethnic status. *Psychiatry Res*. 1990;31(1):69-83. doi:10.1016/0165-1781(90)90110-Q

31. Roberts R, Vernon S. The Center for Epidemiologic Studies Depression Scale: its use in a community sample. *Am J Psychiatry*. 1983;140(1):41-46. doi:10.1176/ajp.140.1.41

32. Simoni JM, Ng MT. Trauma, coping, and depression among women with HIV/AIDS in New York City. *AIDS Care - Psychol Socio-Medical Asp AIDS/HIV*. 2000;12(5):567-580. doi:10.1080/095401200750003752

33. Illumina. Infinium® MethylationEPIC BeadChip. *Illumina Mark*. 2015:4. https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/humanmethylationepic-data-sheet-1070-2015-008.pdf.

34. Bibikova M, Barnes B, Tsan C, et al. Genomics High density DNA methylation array with single CpG site resolution Unmethylated locus Methylated locus Unmethylated locus Methylated locus. *Genomics*. 2011;98(4):288-295. doi:10.1016/j.ygeno.2011.07.007

35. Illumina. Infinium HD Assay Methylation Protocol Guide. 2015;(November). www.illumina.com.

36. Heiss JA, Just AC. Improved filtering of DNA methylation microarray data by detection p values and its impact on downstream analyses. *Clin Epigenetics*. 2019;11(15):1-8.

37. Fortin J, Labbe A, Lemire M, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol*. 2014;15(503):1-17.

38. Jun G, Flickinger M, Hetrick KN, et al. Detecting and Estimating Contamination of Human DNA Samples in Sequencing and Array-Based Genotype Data. *Am J Hum Genet*. 2012;91:839-848. doi:10.1016/j.ajhg.2012.09.004

39. Jiao C, Zhang C, Dai R, et al. Positional effects revealed in Illumina methylation array

and the impact on analysis. *Epigenomics*. 2018;10(5):643-659. doi:10.2217/epi-2017-0105

40. Min JL, Hemani G, Davey Smith G, Relton C, Suderman M. Meffil: efficient normalization and analysis of very large DNA methylation datasets. *Bioinformatics*. 2018;34(23):3983-3989. doi:10.1093/bioinformatics/bty476

41. Leek JT, Storey JD. Capturing Heterogeneity in Gene Expression Studies by Surrogate Variable Analysis. *PLoS Genet*. 2007;3(9):e161. doi:10.1371/journal.pgen.0030161

42. Leek JT, Storey JD. A general framework for multiple testing dependence. *PNAS*. 2008;105(48):18718-18723.

43. Waite LL, Weaver B, Day K, et al. Estimation of Cell-Type Composition Including T and B Cell Subtypes for Whole Blood Methylation Microarray Data. *Front Genet*. 2016;7(February):1-10. doi:10.3389/fgene.2016.00023

44. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13(1). doi:10.1186/1471-2105-13-86

45. Suderman M, Staley JR, French R, Arathimos R, Simpkin A, Tilling K. Dmrff: Identifying Differentially Methylated Regions Efficiently With Power and Control. *bioRxiv*. 2018:508556. doi:10.1101/508556

46. Yin Y, Morgunova E, Jolma A, et al. Impact of cytosine methylation on DNA binding specificities of human transcription factors. *Science (80-)*. 2017;2239(May). doi:10.1126/science.aaj2239

47. Affinito O, Palumbo D, Fierro A, et al. Genomics Nucleotide distance in fl uences co-methylation between nearby CpG sites. *Genomics*. 2020;112:144-150. doi:10.1016/j.ygeno.2019.05.007

48. Saito D, Suyama M. Linkage disequilibrium analysis of allelic heterogeneity in DNA methylation Linkage disequilibrium analysis of allelic heterogeneity in DNA methylation. *Epigenetics*. 2015;10(12):1093-1098. doi:10.1080/15592294.2015.1115176

49. Bonferroni C. *Teoria Statistica Delle Classi e Calcolo Delle Probabilita*. Firenze, Italia: R Instituto Superiore de Scienze Economiche e Commerciali; 1936.

50. Gordon A, Glazko G, Qiu X, Yakovlev A. Control of the mean number of false discoveries, Bonferroni and stability of multiple testing. *Ann Appl Stat*. 2007;1(1):179-190. doi:10.1214/07-AOAS102

51. Diz A, Carvajal-Rodriguez A, Skibinski D. Multiple hypothesis testing in proteomics: A strategy for experimental work. *Mol Cell Proteomics*. 2011;10(3). doi:10.1074/mcp.M110.004374

52. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. *R Stat Soceity*. 1995;57(1):289-300.

53. Pawitan Y, Michiels S, Koscielny S, Gusnanto A, Ploner A. Gene expression False discovery rate , sensitivity and sample size for microarray studies. *Bioinformatics*. 2005;21(13):3017-3024. doi:10.1093/bioinformatics/bti448

54. Staley J, Battram T, Hemani G, Suderman M, Relton C, Guant T. The MRC-IEU catalog of epigenome-wide association studies. EWAS Catalog. http://ewascatalog.org/. Published 2017.

55. Li M, Zou D, Li Z, et al. EWAS Atlas : a curated knowledgebase of epigenome-wide association studies. *Nucleic Acids Res*. 2019;47(October 2018):983-988. doi:10.1093/nar/gky1027

56. Hannon E, Lunnon K, Schalkwyk L, Mill J. Interindividual methylomic variation across blood, cortex, and cerebellum: Implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics*. 2015;10(11):1024-1032. doi:10.1080/15592294.2015.1100786

57. Ressler K, Mercer K, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and PAC1 receptor. *Nature*. 2011;470(7335):492-497. doi:10.1097/CCM.0b013e31823da96d.Hydrogen

58. Miller MW, Maniates H, Wolf EJ, et al. Brain , Behavior , and Immunity CRP polymorphisms and DNA methylation of the AIM2 gene influence associations between trauma exposure , PTSD , and C-reactive protein. *Brain Behav Immun*. 2018;67:194-202. doi:10.1016/j.bbi.2017.08.022

59. Moser DA, Paoloni-Giacobino A, Stenz L, et al. BDNF methylation and maternal brain activity in a violence-related sample. *PLoS One*. 2015;10(12):e0143427.

doi:10.1371/journal.pone.0143427

60. Kim TY, Kim SJ, Chung HG, Choi JH, Kim SH, Kang JI. Epigenetic alterations of the BDNF gene in combat-related post-traumatic stress disorder. *Acta Psychiatr Scand*. 2017;135(2):170-179. doi:10.1111/acps.12675

61. Norrholm SD, Jovanovic T, Smith AK, et al. Differential genetic and epigenetic regulation of catechol-O-methyl-transferase (COMT) is associated with impaired fear inhibition in posttraumatic stress disorder. *Front Behav Neurosci*. 2013;7(MAR):1-10. doi:10.3389/fnbeh.2013.00030

62. Bishop JR, Lee AM, Mills LJ, et al. Methylation of FKBP5 and SLC6A4 in Relation to Treatment Response to Mindfulness Based Stress Reduction for Posttraumatic Stress Disorder. *Front Psychiatry*. 2018;9(September):1-11. doi:10.3389/fpsyt.2018.00418

63. Yehuda R, Daskalakis NP, Bierer LM, et al. Archival Report Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. *Biol Psychiatry*. 2016;80(5):372-380. doi:10.1016/j.biopsych.2015.08.005

64. Kang J, Kim T, Choi J, So H, Kim S. Allele-specific DNA methylation level of FKBP5 is associated with post- traumatic stress disorder. *Psychoneuroendocrinology*. 2019;103(November 2018):1-7. doi:10.1016/j.psyneuen.2018.12.226

65. Schechter DS, Moser DA, Pointet VC, et al. The association of serotonin receptor 3A methylation with maternal violence exposure, neural activity, and child aggression. *Behav Brain Res*. 2017;325:268-277. doi:10.1016/j.bbr.2016.10.009

66. Bam M, Yang X, Zhou J, Ginsberg JP, Leyden Q. Evidence for Epigenetic Regulation of Pro-Inflammatory Cytokines , Interleukin-12 and Interferon Gamma , in Peripheral Blood Mononuclear Cells from PTSD Patients. *J Neuroimmune Pharmacol*. 2016;11:168-181. doi:10.1007/s11481-015-9643-8

67. Uddin M, Galea S, Chang S, Aiello AE, Wildman DE. Gene expression and methylation signatures of MAN2C1 are associated with PTSD. *Dis Markers*. 2011;30:111-121. doi:10.3233/DMA-2011-0750

68. Ziegler C, Wolf C, Schiele MA, et al. Monoamine Oxidase A Gene Methylation and Its Role in Posttraumatic Stress Disorder: First Evidence from the South Eastern

Europe (SEE)-PTSD Study. *Int J Neuropsychopharmacol*. 2018;21(5):423-432. doi:10.1093/ijnp/pyx111

69. Labonté B, Azoulay N, Yerko V, Turecki G, Brunet A. Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder. 2014;(November 2013). doi:10.1038/tp.2014.3

70. Schechter DS, Moser DA, Paoloni-Giacobino A, et al. Methylation of NR3C1 is related to maternal PTSD, parenting stress and maternal medial prefrontal cortical activity in response to child separation among mothers with histories of violence exposure. *Front Psychol*. 2015;6(May):1-12. doi:10.3389/fpsyg.2015.00690

71. Schür RR, Boks MP, Rutten BPF, et al. Longitudinal changes in glucocorticoid receptor exon 1F methylation and psychopathology after military deployment. *Transl Psychiatry*. 2017;7:e1181. doi:10.1038/tp.2017.150

72. Vukojevic V, Kolassa I, Fastenrath M, et al. Epigenetic Modification of the Glucocorticoid Receptor Gene Is Linked to Traumatic Memory and Post-Traumatic Stress Disorder Risk in Genocide Survivors. *J Neurosci*. 2014;34(31):10274-10284. doi:10.1523/JNEUROSCI.1526-14.2014

73. Yehuda R, Nikolaos P, Koch E, et al. Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. *Front Psychiatry*. 2013;4(September):1-14. doi:10.3389/fpsyt.2013.00118

74. Mcnerney MW, Sheng T, Nechvatal JM, et al. Integration of neural and epigenetic contributions to posttraumatic stress symptoms : The role of hippocampal volume and glucocorticoid receptor gene methylation. *PLoS One*. 2018;13(2):1-14.

75. Yehuda R, Flory JD, Bierer LM, et al. Lower Methylation of Glucocorticoid Receptor Gene Promoter 1F in Peripheral Blood of Veterans with Posttraumatic Stress Disorder. *Biol Psychiatry*. 2015;77(4):356-364. doi:10.1016/j.biopsych.2014.02.006

76. Nawijn L, Krzyzewska IM, van Zuiden M, et al. Oxytocin receptor gene methylation in male and female PTSD patients and trauma-exposed controls. *Eur Neuropsychopharmacol*. 2019;29(1):147-155. doi:10.1016/j.euroneuro.2018.10.006

77. Sadeh N, Spielberg J, Logue M, et al. SKA2 Methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed

veterans. *Mol Psychiatry*. 2016;21(3):357-363. doi:10.1038/mp.2015.134.SKA2

78. Boks MP, Rutten BPF, Geuze E, et al. SKA2 Methylation is Involved in Cortisol Stress Reactivity and Predicts the Development of Post-Traumatic Stress Disorder (PTSD) After Military Deployment. *Neuropsychopharmacoly*. 2016;41:1350-1356. doi:10.1038/npp.2015.286

79. Chang SC, Koenen KC, Galea S, et al. Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. *PLoS One*. 2012;7(6):1-6. doi:10.1371/journal.pone.0039184

80. Koenen K, Uddin M, Chang S, et al. SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. *Depress Anxiety*. 2011;28:639-647. doi:10.1002/da.20825

81. Smith A, Ratanatharathorn A, Maihofer A, et al. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies novel methylation loci. *bioRxiv Prepr*. 2019. doi:https://doi.org/10.1101/585109

82. Smith A, Conneely K, Kilaru V, et al. Differential immune system DNA methylation and cytokine regulation in Post-Traumatic Stress Disorder. *Am J Med Genet Paert B Neuropsychiatr Genet*. 2011;156B(6):700-708. doi:10.1109/TMI.2012.2196707.Separate

83. Mehta D, Bruenig D, Carrillo-Roa T, et al. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatr Scand*. 2017;136(5):493-505. doi:10.1111/acps.12778

84. Snijders C, Maihofer AX, Ratanatharathorn A, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clin Epigenetics*. 2020;12(1):1-13. doi:10.1186/s13148-019-0798-7

85. Rutten BPF, Vermetten E, Vinkers CH, et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry*. 2018;23(5):1145-1156. doi:10.1038/mp.2017.120

86. Logue MW, Miller MW, Wolf EJ, et al. An epigenome-wide association study of

posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clin Epigenetics*. 2020;12(1):1-14. doi:10.1186/s13148-020-0820-0

87. Maddox SA, Kilaru V, Shin J, et al. Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. *Mol Psychiatry*. 2018;23(3):658-665. doi:10.1038/mp.2016.250

88. Uddin M, Ratanatharathorn A, Armstrong D, Kuan P, Allison E. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics*. 2018;10(12):1585-1601.

89. Claus R, Wilop S, Hielscher T, et al. A systematic comparison of quantitative high-resolution DNA methylation analysis and methylation-specific PCR. *Epigenetics*. 2012;7(7):772-780. doi:10.4161/epi.20299

90. Ehrich M, Nelson MR, Stanssens P, et al. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry. *Proc Natl Acad Sci U S A*. 2005;102(44):15785-15790. doi:10.1073/pnas.0507816102

CHAPTER 5: SUPPLEMENTARY MATERIAL

Supplementary tables for the main effect of *FKBP5* methylation

Supplementary Table 1: *CpG1 methylation levels and covariates predicting PTSD at 3-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG1 (3-months)	-0.68	0.32	-2.14	.035*	-1.32	-0.05
rs1360780	6.38	6.47	0.99	.327	-6.50	19.26
Childhood trauma (baseline)	1.58	0.95	1.66	.102	-0.32	3.47
Depression (baseline)	-0.10	0.24	-0.42	.678	-0.57	0.38
Alcohol consumption (baseline)	-0.89	1.28	-0.70	.487	-3.43	1.65

* p < .05

Supplementary Table 2: *CpG1 methylation levels and covariates predicting PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG1 (6-months)	-0.04	0.30	-0.14	.889	-0.65	0.56
rs1360780	7.69	6.71	1.15	.255	-5.67	21.04
Childhood trauma (baseline)	0.46	0.94	0.49	.628	-1.42	2.34
Depression (baseline)	0.55	0.24	2.24	.028*	0.06	1.04
Alcohol consumption (baseline)	-2.07	1.32	-1.57	.121	-4.71	0.56

* p < .05

Supplementary Table 3: *CpG2 methylation levels and covariates predicting PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG2 (6-months)	-0.15	0.42	-0.36	.718	-0.99	0.69
rs1360780	7.85	6.60	1.19	.238	-5.29	21.00
Childhood trauma (baseline)	0.41	0.93	0.44	.664	-1.45	2.26
Depression (baseline)	0.55	0.24	2.27	.026*	0.07	1.04
Alcohol consumption (baseline)	-2.03	1.32	-1.53	.130	-4.66	0.61

* p < .05

Supplementary Table 4: *CpG5 methylation levels and confounding variables predicting PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG5 (6-months)	-0.82	0.36	-2.29	.025*	-1.53	-0.11
rs1360780	5.98	6.53	0.92	.363	-7.02	18.97
Childhood trauma (baseline)	0.39	0.90	0.43	.668	-1.40	2.17
BMI (baseline)	-0.04	0.53	-0.07	.945	-1.10	1.02
Medication use (baseline)	14.12	6.89	2.05	.044*	0.40	27.83

* p < .05
Abbreviations: body mass index (BMI)

Supplementary Table 5: *CpG5 methylation levels and covariates predicting PTSD at 6-months post-rape*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG5 (6-months)	-0.70	0.35	-1.98	.051	-1.40	0.00
rs1360780	7.04	6.36	1.11	.272	-5.62	19.70
Childhood trauma (baseline)	0.31	0.90	0.34	.734	-1.49	2.11
BMI (baseline)	-0.34	0.53	-0.65	.521	-1.40	0.72
Medication use (baseline)	14.06	6.78	2.07	.042*	0.55	27.57
Depression (baseline)	0.47	0.24	1.92	.059	-0.02	0.95
Alcohol consumption (baseline)	-2.28	1.30	-1.76	.083	-4.86	0.30

* p < .05
Abbreviations: body mass index (BMI)

Supplementary tables for the main effect of childhood trauma

Supplementary Table 6: *Childhood trauma and covariates (including CpG1 methylation) predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG1 (baseline)	-0.01	0.19	-0.03	.977	-0.37	0.36
rs1360780	-2.58	4.17	-0.62	.539	-10.89	5.73
Childhood trauma (baseline)	1.66	0.70	2.37	.021*	0.26	3.05
Depression (baseline)	0.67	0.15	4.52	.00002*	0.38	0.97
Alcohol consumption (baseline)	-1.68	0.83	-2.02	.047*	-3.34	-0.02

* p < .05

Supplementary Table 7: *Childhood trauma and covariates (including CpG2 methylation) predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG2 (baseline)	-0.33	0.24	-1.40	.166	-0.80	0.14
rs1360780	-3.67	4.09	-0.90	.372	-11.81	4.47
Childhood trauma (baseline)	1.43	0.68	2.08	.041*	0.06	2.79
Depression (baseline)	0.63	0.14	4.43	.00003*	0.35	0.92
Alcohol consumption (baseline)	-1.05	0.84	-1.25	.215	-2.72	0.62

* p < .05

Supplementary Table 8: *Childhood trauma and covariates (including CpG3 methylation) predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG3 (baseline)	-0.22	0.18	-1.21	.230	-0.58	0.14
rs1360780	-2.37	4.07	-0.58	.563	-10.48	5.74
Childhood trauma (baseline)	1.68	0.69	2.43	.018*	0.30	3.05
Depression (baseline)	0.68	0.15	4.67	.00001*	0.39	0.97
Alcohol consumption (baseline)	-1.70	0.81	-2.09	.040*	-3.33	-0.08

* p < .05

Supplementary Table 9: *Childhood trauma and covariates (including CpG4 methylation) predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG4 (baseline)	-0.40	0.28	-1.44	.154	-0.95	0.15
rs1360780	-2.84	4.04	-0.70	.485	-10.88	5.21
Childhood trauma (baseline)	1.62	0.69	2.34	.021*	0.25	2.99
Depression (baseline)	0.69	0.14	4.77	.000008*	0.40	0.98
Alcohol consumption (baseline)	-1.71	0.81	-2.11	.038*	-3.32	-0.10

* $p < .05$

Supplementary Table 10: *Childhood trauma and covariates (including CpG5 methylation) predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG5 (baseline)	0.07	0.23	0.30	.765	-0.38	0.52
rs1360780	-2.58	4.19	-0.62	.539	-10.93	5.76
Childhood trauma (baseline)	1.66	0.70	2.36	.021*	0.26	3.05
Depression (baseline)	0.68	0.15	4.62	.00002*	0.39	0.98
Alcohol consumption (baseline)	-1.64	0.82	-1.99	.050	-3.28	0.00

* $p < .05$

Supplementary table for the interaction effect between methylation and childhood trauma

Supplementary Table 11: *Comparison of correlations between methylation and PTSD for the groups with low vs high childhood trauma*

	n	%	*r*	*p*	*PTSD*		*z*	*p*
					M	*SD*		
CpG1 & PTSD (baseline)	84	100					-0.36	.359
Low childhood trauma	42	50	-.132	.405	61.96	3.06		
High childhood trauma	42	50	-.051	.750	71.17	3.06		
CpG2 & PTSD (baseline)	84	100					-0.20	.419
Low childhood trauma	42	50	-.189	.230	62.06	2.93		
High childhood trauma	42	50	-.144	.365	71.53	2.94		
CpG3 & PTSD (baseline)	84	100					0.64	.263
Low childhood trauma	42	50	-.033	.835	61.77	3.07		
High childhood trauma	42	50	-.175	.269	71.23	3.07		
CpG4 & PTSD (baseline)	85	100					0.54	.295
Low childhood trauma	43	50.6	.002	.990	61.66	3.05		
High childhood trauma	42	49.4	-.119	.451	71.17	3.08		
CpG5 & PTSD (baseline)	85	100					0.59	.277
Low childhood trauma	43	50.6	.069	.658	61.62	3.05		
High childhood trauma	42	49.4	-.064	.688	71.37	3.09		

Supplementary tables for the interaction effect between methylation, rs1360780 genotype and childhood trauma

Supplementary Table 12: *Correlation between methylation and PTSD: comparison of childhood trauma and genotype subgroups*

	N	%	*r*	*p*	*PTSD*		*z*	*p*
					M	*SD*		
CpG1 & PTSD (baseline)	42	100					0.32	.376
Low childhood trauma & CC genotype	14	33.3	-.040	.892	65.82	5.33		
Low childhood trauma & CT/TT genotype	28	66.7	-.153	.436	59.88	3.77		
CpG1 & PTSD (baseline)	41	100					-0.40	.345
High childhood trauma & CC genotype	13	31.7	-.187	.541	72.51	5.68		
High childhood trauma & CT/TT genotype	28	68.3	-.040	.838	70.04	3.79		
CpG2 & PTSD (baseline)	43	100					0.90	.183
Low childhood trauma & CC genotype	14	32.6	.180	.557	67.87	5.94		
Low childhood trauma & CT/TT genotype	29	67.4	-.142	.462	59.77	3.52		
CpG2 & PTSD (baseline)	41	100					-1.00	.160
High childhood trauma & CC genotype	13	31.7	-.434	.138	74.75	5.26		
High childhood trauma & CT/TT genotype	28	68.3	-.092	.641	69.66	3.56		
CpG3 & PTSD (baseline)	42	100					0.61	.271
Low childhood trauma & CC genotype	14	33.3	.180	.538	65.24	5.40		
Low childhood trauma & CT/TT genotype	28	66.7	-.039	.846	60.04	3.80		
CpG3 & PTSD (baseline)	41	100					-8.70	.000*
High childhood trauma & CC genotype	13	31.7	-.575	.040*	74.30	5.58		
High childhood trauma & CT/TT genotype	28	68.3	-.003	.989	69.37	3.79		
CpG4 & PTSD (baseline)	43	100					-0.01	.497
Low childhood trauma & CC genotype	14	32.6	.031	.916	65.62	5.34		
Low childhood trauma & CT/TT genotype	29	67.4	.034	.861	59.58	3.74		
CpG4 & PTSD (baseline)	41	100					0.40	.345
High childhood trauma & CC genotype	13	31.7	-.075	.808	74.09	5.54		
High childhood trauma & CT/TT genotype	28	68.3	-.221	.259	69.22	3.80		
CpG5 & PTSD (baseline)	43	100					1.40	.081
Low childhood trauma & CC genotype	14	32.6	.347	.224	65.84	5.35		
Low childhood trauma & CT/TT genotype	29	67.4	-.139	.471	59.21	3.73		
CpG5 & PTSD (baseline)	41	100					-5.89	.000*
High childhood trauma & CC genotype	13	31.7	-.825	.001**	73.87	5.72		
High childhood trauma & CT/TT genotype	28	68.3	.057	.774	69.60	3.79		
CpG1 & PTSD (6-months)	42	100					-0.98	.164
Low childhood trauma & CC genotype	14	33.3	-.362	.204	21.05	7.70		
Low childhood trauma & CT/TT genotype	28	66.7	-.026	.894	26.70	5.95		
CpG1 & PTSD (6-months)	41	100					0.10	.461
High childhood trauma & CC genotype	13	31.7	.117	.703	20.54	8.16		
High childhood trauma & CT/TT genotype	28	68.3	.081	.681	29.57	5.71		
CpG2 & PTSD (6-months)	42	100					0.17	.432
Low childhood trauma & CC genotype	13	31.0	-.066	.829	20.22	7.74		
Low childhood trauma & CT/TT genotype	29	69.0	-.129	.505	27.95	6.20		
CpG2 & PTSD (6-months)	41	100					-1.27	.103
High childhood trauma & CC genotype	13	31.7	-.165	.590	23.10	7.73		
High childhood trauma & CT/TT genotype	28	68.3	.298	.124	28.25	5.70		
CpG3 & PTSD (6-months)	41	100					1.44	.075

Low childhood trauma & CC genotype	13	31.7	.382	.178	20.54	7.99		
Low childhood trauma & CT/TT genotype	28	68.3	-.136	.489	26.54	5.98		
CpG3 & PTSD (6-months)	41	100					-1.36	.087
High childhood trauma & CC genotype	13	31.7	-.047	.878	23.05	7.82		
High childhood trauma & CT/TT genotype	28	68.3	.432	.022*	28.64	5.66		
CpG4 & PTSD (6-months)	43	100					-0.13	.448
Low childhood trauma & CC genotype	14	32.6	.196	.503	19.66	7.77		
Low childhood trauma & CT/TT genotype	29	67.4	.241	.207	26.44	5.96		
CpG4 & PTSD (6-months)	41	100					-0.80	.212
High childhood trauma & CC genotype	13	31.7	-.269	.375	23.87	7.76		
High childhood trauma & CT/TT genotype	28	68.3	.023	.909	29.77	5.74		
CpG5 & PTSD (6-months)	43	100					1.80	.036*
Low childhood trauma & CC genotype	14	32.6	.316	.271	23.05	7.62		
Low childhood trauma & CT/TT genotype	29	67.4	-.309	.103	26.80	5.82		
CpG5 & PTSD (6-months)	41	100					-0.04	.483
High childhood trauma & CC genotype	13	31.7	-.140	.648	20.19	7.64		
High childhood trauma & CT/TT genotype	28	68.3	-.124	.530	28.59	5.50		

* $p < .05$

Supplementary Table 13: *Methylation, rs1360780 genotype and childhood trauma interaction with covariates predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG3 (baseline) *rs1360780*childhood trauma	-0.00	0.00	-0.39	.698	-0.01	0.00
Depression (baseline)	0.72	0.15	4.87	.000006*	0.43	1.02
Alcohol consumption (baseline)	-1.14	0.82	-1.40	.167	-2.77	0.49

* p < .05

Supplementary Table 14: *Methylation, rs1360780 genotype and childhood trauma interaction with confounding variables predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG5 (baseline) *rs1360780*childhood trauma	0.00	0.00	-0.58	.564	-0.01	0.01
BMI (baseline)	0.29	0.37	0.80	.428	-0.44	1.03
Medication use (baseline)	-5.88	4.60	-1.28	.205	-15.04	3.28

* p < .05
Abbreviations: body mass index (BMI)

Supplementary Table 15: *Methylation, rs1360780 genotype and childhood trauma interaction with covariates predicting PTSD at baseline*

	ß	Std error	*t*	*p*	95% CI Lower	95% CI Upper
CpG5 (baseline) *rs1360780*childhood trauma	0.00	0.00	-0.15	.885	-0.01	0.01
BMI (baseline)	-0.03	0.33	-0.09	.925	-0.69	0.63
Medication use (baseline)	-6.03	4.08	-1.48	.143	-14.15	2.09
Depression (baseline)	0.74	0.15	4.90	.000005*	0.44	1.04
Alcohol consumption (baseline)	-0.98	0.82	-1.20	.235	-2.62	0.65

* p < .05
Abbreviations: body mass index (BMI)

ADDENDUM A

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

Approval Notice

Response to Modifications- (New Application)

09-Dec-2016
Nothling, Jani J

Ethics Reference #: S16/08/146
Title: Epigenomic analysis of posttraumatic stress disorder in female rape survivors in South Africa

Dear Miss Jani Nothling,

The **Response to Modifications** - ***(New Application)*** received on **30-Nov-2016**, was reviewed by members of **Health Research Ethics Committee 2** via Expedited review procedures on **09-Dec-2016** and was approved.
Please note the following information about your approved research protocol:

Protocol Approval Period: **09-Dec-2016 -08-Dec-2017**

Please remember to use your **protocol number** (**S16/08/146**) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB 0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics

approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at .

Included Documents:

6.2. Addenda, participant information and consent - Jani Nothling.pdf
9.2. PhD evaluation committee final report - Ms J Nothling.pdf
20161130 MOD Addendum C2 Genetics Informed Consent
9.1. PhD evaluation committee checklist - J Nothling.pdf
20161130 MOD Fully Executed SLA signed on 21112016
7.1. - CV - Jani Nothling.pdf
20161130 MOD Consent form
20161130 MOD Addendum C4 RICE HIV ICF
4. PhD protocol.pdf
20161130 MOD Addendum C1 Biometric Consent
1. HREC application form.pdf
5. Protocol synopsis.pdf
20161130 MOD SU HREC Acknowledgement Letter
20161130 MOD Addendum G MRC main study Ethics appr
7.3. - CV - Soraya Seedat.pdf
8.3. Investigator declaration - Naeemah Abrahams sign.pdf
7.2. - CV - Sian Hemmings.pdf
8.1. Investigator declaration - Jani Nothling.pdf
20161130 MOD Response to ethics comments
20161130 MOD Protocol synopsis
2. HREC Checklist.pdf
0. Cover Letter.pdf
6.1. Addenda Index.pdf
7.4. - CV - Naeemah Abrahams.pdf
8.2. Investigator declaration - Sian Hemmings12.pdf
8.4. Investigator declaration - Soraya Seedat 1.pdf
3. HREC PaymentInstruction_Health research.pdf

Sincerely,

Francis Masiye
HREC Coordinator
Health Research Ethics Committee 2

Investigator Responsibilities

Protection of Human Research Participants

Some of the responsibilities investigators have when conducting research involving human participants are listed below:

1.Conducting the Research. You are responsible for making sure that the research is conducted according to the HREC approved research protocol. You are also responsible for the actions of all your co-investigators and research staff involved with this research.

2.Participant Enrolment. You may not recruit or enrol participants prior to the HREC approval date or after the expiration date of HREC approval. All recruitment materials for any form of media must be approved by the HREC prior to their use. If you need to recruit more participants than was noted in your HREC approval letter, you must submit an amendment requesting an increase in the number of participants.

3.Informed Consent. You are responsible for obtaining and documenting effective informed consent using **only** the HREC-approved consent documents, and for ensuring that no human participants are involved in research prior to obtaining their informed consent. Please give all participants copies of the signed informed consent documents. Keep the originals in your secured research files for at least fifteen (15) years.

4.Continuing Review. The HREC must review and approve all HREC-approved research protocols at intervals appropriate to the degree of risk but not less than once per year. There is **no grace period.** Prior to the date on which the HREC approval of the research expires, **it is your responsibility to submit the continuing review report in a timely fashion to ensure a lapse in HREC approval does not occur.** If HREC approval of your research lapses, you must stop new participant enrolment, and contact the HREC office immediately.

5.Amendments and Changes. If you wish to amend or change any aspect of your research (such as research design, interventions or procedures, number of participants, participant population, informed consent document, instruments, surveys or recruiting material), you must submit the amendment to the HREC for review using the current Amendment Form. You **may not initiate** any amendments or changes to your research without first obtaining written HREC review and approval. The **only exception** is when it is necessary to eliminate apparent immediate hazards to participants and the HREC should be immediately informed of this necessity.

6.Adverse or Unanticipated Events. Any serious adverse events, participant complaints, and all unanticipated problems that involve risks to participants or others, as well as any research-related injuries, occurring at this institution or at other performance sites must be reported to the HREC within **five (5) days** of discovery of the incident. You must also report any instances of serious or continuing problems, or non-compliance with the HRECs requirements for protecting human research participants. The only exception to this policy is that the death of a research participant must be reported in accordance with the Stellenbosch Universtiy Health Research Ethics Committee Standard Operating Procedures www.sun025.sun.ac.za/portal /page/portal/Health_Sciences/English/Centres%20and%20Institutions/Research_Development_Support/Ethics/Application_package All reportable events should be submitted to the HREC using the Serious Adverse Event Report Form.

7.Research Record Keeping. You must keep the following research-related records, at a minimum, in a secure location for a minimum of fifteen years: the HREC approved research protocol and all amendments; all informed consent documents; recruiting materials; continuing review reports; adverse or unanticipated events; and all correspondence from the HREC

8.Reports to the MCC and Sponsor. When you submit the required annual report to the MCC or you submit required reports to your sponsor, you must provide a copy of that report to the HREC. You may submit the report at the time of continuing HREC review.

9.Provision of Emergency Medical Care. When a physician provides emergency medical care to a participant without prior HREC review and approval, to the extent permitted by law, such activities will not be recognised as research nor will the data obtained by any such activities should it be used in support of research.

10.Final reports. When you have completed (no further participant enrolment, interactions, interventions or data analysis) or stopped work on your research, you must submit a Final Report to the HREC.

11.On-Site Evaluations, MCC Inspections, or Audits. If you are notified that your research will be reviewed or audited by the MCC, the sponsor, any other external agency or any internal group, you must inform the HREC immediately of the impending audit/evaluation.

ADDENDUM B

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

ETHICS COMMITTEE

PO Box 19070, 7505 Tygerberg, South Africa
Francie van Zijl Drive, Parowvallei, 7500
Tel: +27 (0)21 938-0687; Fax: +27 (0) 866-854023
E-mail: adri.labuschagne@mrc.ac.za
http://www.mrc.ac.za/ethics/ethics.htm

28 July 2014

Prof N Abrahams
Gender and Health Research Unit
MRC Cape Town

Dear Prof Abrahams

Protocol ID: EC019-10/2013
Protocol title: The impact of rape in women on HIV acquisition and retention and linkages to care: a longitudinal study
Meeting date: 24 June 2014

Thank you for your application to the Committee for an amendment, dated 13 May 2014, and your responses dated 11 June 2014 and 9 July 2014. The responses were found to be acceptable. I am pleased to inform you that ethics approval is now granted for the amendment.

Wishing you well with your research.

Yours sincerely

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

ADDENDUM C

A STUDY ON WOMEN'S HEALTH AND WELL-BEING

INFORMED CONSENT

RECRUITED AT _____

RICE - WOMEN'S HEALTH AND WELLBEING STUDY

INTRODUCTION

Hello, welcome to our clinic. You are being invited to take part in the women's health and well-being study. It is a study that the Medical Research Council is conducting with women in Durban on women's health and wellbeing. We want to learn more about women's health so we can help the Government in better planning of their services so that they can meet women's health needs.

We also want to have a better understanding about women's physical and mental health problems, how long these problems last, what causes them, and what social factors and life experiences affect them, whether the problems persist and if the problems require treatment. We are also very interested to know how experiences of violence affect women's health. In addition, we want to get a better understanding on how women heal after being hurt.

This is an information and consent form, and we are inviting women between the ages of 18 – 40 years who attend Thuthuzela Care Centre/Rape Centres after they have been sexual assaulted. One of the primary reasons for doing this research is to understand the health problems that women who have been raped experience. Your participation in our research will not influence the health care that you receive at the Thuthuzela Care Centre/Rape Centres or your case. We will not ask you questions about the rape experience or the circumstances of the incident. We will ask about other sexual violence experiences. We understand that these questions might bother you and we will stop and can always ask these same questions at a later interview. However, we will also be prepared to help you if you need to speak to someone.

This study will be done over a period of three years and we will speak to women on repeat occasions. This means that you will be invited to come to the research clinic and have interviews done every 3 months. At each study visit we will ask you some interview questions, we will also ask you to give us permission to do some blood tests and tests on your urine as well as to provide a swab. We will explain more about this later. This means we will have to remain in contact so that we can invite you to return for the follow-up interviews over the three years.

IS MY PARTICIPATION VOLUNTARY IN THIS STUDY

This form gives you information about the study and what will be expected of you. Once you have read and understood and agree to participate, you will then be asked to sign your name on this form. You will be given a copy of this form to keep. It is important that you know that your participating is voluntary, and you are free to withdraw from the study at any point, even after you have agreed to take part.

WHAT HAPPENS IF I DECIDE TO PARTICPATE IN THE STUDY?

If you agree to participate in the study, then we will start with your baseline visit today. You will see and talk to both of us (nurse and research assistant) today. We will start by explaining in more detail

about the procedures that will take place today and we will answer questions before you sign the informed consent for us to continue. The nurse will see you first and will do health assessments including the understanding of how you cope, taking of your blood pressure, your pulse, your weight and provide advice on healthy living. You will then be seen by the other researcher and she will ask questions about yourself, your relationships with husbands and boyfriends and other health questions sexual and reproductive health matters, as well as questions about experiences of violence. There will be some questions about things which are often thought of as secrets. Let us know if you feel uncomfortable about these questions. At the end the nurse will take blood, urine and hair samples and will give you the answers to the tests as soon as we get them and will discuss your health with you. Everything that you tell the interviewer will be kept secret and you have the right not to answer any questions that you do not wish to answer. The answers to the questions will be entered on a computer and we expect the interviews with the two of us to take about 2 hours. We will also refer you to the health clinics if we think it is necessary.

WHAT ARE THE PROCEDURES IN THIS STUDY?

If you are interested in being part of this study, the following will take place:

The Research assistant start by using a biometric system to log her on the study data entry system. A biometric system allows researchers to use her fingerprint to identify her every time she returns to the clinic. Her fingerprint will be linked to a number and this number will be used to store the information and this will ensure her information remains safe and confidential. The reason why we need to identify her fingerprint each time she visit the clinic is the study is over a long period of time, the patients' information can get mixed up so by entering your details with your study number it prevents your information getting mixed up with another participant's information. ***We will ask you to give us permission to use the biometric system in a separate informed consent that you will sign***.

A study nurse will do a mental health assessment as well as a screen for high blood pressure as we explained earlier.

The research assistant will then do an interview asking the questions as we explained earlier, and she will enter this on the computer as she speaks with you.

Hair sample- We will ask you to allow us to collect about 6-8 strands of hair between 3-6 cm. We will take it from the back of the head, close to the scalp and we will do it at each visit. We aware that this might upset your hairstyle and we have a person here that will ensure that your hair is not disarranged. She will make sure that your hair looks exactly the same again after we taken the hair sample. The hair samples will be sent to a special laboratory in Germany where they will be examined and will provide us information about levels of stress we experience. The reason why it must be done in Germany is because we in South Africa do not have the machines and the equipment to examine it here. Because our hair grows slowly it will allow us to examine the levels of stress over the previous 2 months. Finding the levels of stress is very useful as it will assist us in coping better with stressful events in our lives. We will provide you with the test result as soon as we get it. We will be very careful about how we take the hair. In order to take a hair sample, the following will be important:

- Your hair must be longer than 3 cm
- You must not have any signs of hair loss or baldness
- You do not have had a severe physical disease in the last 5 years
- You have not been taking anti-depressants in the last 6 months

We will then collect some samples to screen your health as part of the study to answer the questions we have about women's health at each time when you are interviewed. The following are tests and samples that we plan to collect at the screening visit as well as all study visits thereafter:

- **Urine test**- we will require a sample of your urine to test for pregnancy. We will give you the result as soon as it has been tested in the lab here at the clinic. This test will be done every time you visit.
- **Swab from your vulva** – this is to test for infections passed through sex. This test is done by taking a smear from vagina. This is similar to a pap smear test, but we do not use instruments and the nurse will assist you and show you how to do it. You will do it in private. The results will be given and discussed with you as soon as it comes back from the laboratory. This test will be done every time you visit.
- **Blood tests-** Blood will be collected from you for various tests. This will be done by the nurse and we will collect about 4-5 teaspoons of blood at each study visit.

We will collect the blood for the following:

- **To test for diabetes, cholesterol levels, kidney and heart diseases in blood.** Women who experience stress and mental problems may be more likely to develop heart cardiovascular disease risk factors such as diabetes, high cholesterol and related kidney problems. We would like to test for diabetes and cholesterol (fat) levels in blood as well as any related kidney problems. We would also like to test for other indicators (signs) of heart disease in the blood. This is a very common illness and it is often hidden. We will also test for fat in your blood which can contribute to heart disease. We will also give you the test results once we receive it from the laboratory. This test only be done on 3 of the visits.
- **To test for infections that passed through sex, including herpes infection and HIV**. We will inform you of your STI test results and whether any treatment is needed. If treatment is needed, we will assist you in receiving treatment.
- **HIV test:** you will receive counselling before the HIV test and also after the test. You will be told the HIV result as soon as it is available. The nurse will speak to you about the meaning of the results, how you feel, and ways to prevent HIV and other sexually transmitted infections. Sometimes the HIV test is not clear and in this case, we will do an additional test until we confirm your results for sure. We will discuss the repeat test with you and what this means. The result will be kept secret. The information will be kept by the Medical Research Council and the people who see it will not know your name. The information will not be given to anyone else who may come to learn that we have it. ***We will ask you to give us permission to do the HIV test at each study visit in a separate informed consent.*** This test will be done every 3 months.
- If you are HIV positive, we will also do a CD4 test. By measuring the CD4 cells in your body we can determine the progression of the HIV. CD4 cells are cells that give us information on how well our immune system is working and they tell us when a person should start with anti-retroviral therapy (HIV treatment). If you are on HIV treatment, we will also measure the viral load as this tells us how well the treatment is working. We will measure these every six months during the research, and we will give you the outcome of the test result and help you to understand what it means. We will also refer you to your local health care clinic to get the necessary treatment. This test will be done at each visit depending on if you are HIV positive.
- **P24 Antigen Test**: If you seroconvert during the study- this means you become HIV positive during the period of this study then we will do a special test called a p24 antigen test which

will tell us information on how long ago the infection happened. This May mean that we have to prick you again as we only know that your HIOV status has changed after testing the blood.

- **Herpes test:** We will also test the blood for herpes (1 teaspoon), which is a virus that can cause you to have painful ulcers around your vagina, although in many people who are infected there is little pain. This is an important virus as people who have it are at greater risk of catching HIV if they have sex with a person who is HIV positive. We want to test to see if you have this. We will give you the test result as soon as we get it back from the laboratory and we will help you to understand the result. This test will be done every time you visit.

- **Genetic research** - We will take a blood sample (about 2 teaspoons) to examine the genes in your blood.
  What is Genetic research? Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like, such as the colour of our eyes or how tall we will grow. Sometimes, genes also give us information about what kind of diseases we may be susceptible to, such as heart disease or diabetes. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions. Some factors in our environment can bring about changes in our genes and we are particularly interested to know about changes in the genes that will affect our ability to cope***. We will ask you to give us permission to take a sample of blood for genetic testing at each study visit in a separate informed consent.*** This test will not be done on every visit.

- **Storage of left-over specimens –** If you agree to participate in the study and agree to have the above blood specimens taken we will ask you to allow us to store the left over samples such as left over blood. These left-over samples might be useful for future related studies and we will use it to look for additional evidence of disease progression. For example, we may further look at your genes. We will store this in a safe place called a repository. Nobody will have access to this left-over blood sample other than the study researchers and it will not be used unless the blood can be used in a study approved by the Ethics committee. We plan to keep this left-over blood sample for 10 years and it will be destroyed after 10 years if it is not used. There are no names on any of the specimens, only a special study number. The people who run the repository and the scientists who later use the specimens will not know your name or any other information about you that might identify you. You may decide that you do not want your left-over blood samples stored for future research studies. You can still participate in this study even if you make this decision, any leftover specimens from you will be destroyed at the end of the study. People always have the right to stop participating in research. So, if you decide that you do not want researchers to be able to use the left-over specimens in the repository, you can contact the clinic staff. They will tell the repository that the specimens with your study code number should not be studied, and these specimens will be destroyed. There are no direct benefits to you from storing your left-over specimens. You may be helping people in the future from the results of studies using the stored specimens. All these specimens are being collected as part of the RICE study in which you are participating. We are not asking you to give any additional specimens for storage, so there is no additional risk associated with collection. The specimens are stored only by code number (not your name) so there is no risk of loss of privacy.

Will you get told your results?

You will not normally get the result of the genetic testing as these tests are experimental. Also, the blood samples from all participants will be de-identified and analysed together and not individually. While the genetic test will not benefit you directly, the research may benefit people who have experienced a traumatic event in the future. In addition, the tests done on the samples of blood stored may not be ready for many years. Your personal results will be made known to you only under special circumstances and **only if they indicate** that you may:

- have a definite risk for developing a particular disorder
- have a condition or predisposition to developing a condition that is treatable or avoidable, e.g. by a lifestyle modification
- need genetic counseling.

When this happens, you will be supported by the staff at the genetic clinic who are experts in helping people understand these diseases. It is however very unlikely that this will happen as such diseases are rare.

The same apply for results from tests done on the stored blood samples. If the researcher's findings could provide important information for your medical care, then they will contact the research staff at your site with the results, and the staff at your clinic can link the code with your name and notify you of the results. If you would like to be contacted with this sort of information, you must notify the study staff of any changes in your address or phone number.

We will ask you to give us permission to store the blood at each study visit in a separate informed consent.

WHAT ARE THE BENEFITS TO ME IF I PARTICIPATE IN THIS STUDY

The main benefit will be in gaining information about your physical and mental health. If we detect that you have any mental or physical health problems, with your permission, we will refer you to your local health care facility for treatment. It is very beneficial for you to learn your HIV status, if you are negative you can make decisions to protect yourself to make sure you remain negative. If you are positive it is important to know so you can get on treatment as soon as you need it and can look after yourself. If you have sexually transmitted infections, we will find this out on the day of your visit and we will treat you for this infection, it is important to know so these can be treated as otherwise you may develop other health problems or become infertile. It is also very useful to know early if you are pregnant so you can receive early care.

Benefits for society

It is very important for South Africa women that we understand their health better and can plan health services that can properly meet their physical and mental health needs. In order to do this effectively it is necessary to have information about what places at women at risk of health problems and how great this risk is. By participating in this study, you will be playing an important role in helping us build this knowledge.

WHAT ARE THE RISKS?

There are no major medical risks involved with participating in this study. Some of the questions may make you feel sad, especially when we ask about experiences of violence, but the study staff will give you support if this happens. There should not be a risk that your private information becomes known to anyone because the study staff will use a special project ID number to identify you and the information you provide. All the staff involved in this study have been given special training on the importance of confidentiality. Some of the women in the study have been invited for

the interviews because they experienced trauma and violence in their lives and some of them have not. Only the interviewer will know whether you have been invited because of violence in your life, no one else will know this.

WHAT ARE THE COSTS TO ME?

There is no cost to you for participating in this study. Y You will be given R80 at the baseline visit and at every visit thereafter you will receive an increment of R20 to compensate for time spent completing the questionnaire and giving the samples. You will also be given money to cover travel costs for each of the visits and we will provide you with a snack every time you come for the interviews.

WHAT IF I CHANGE MY MIND AFTER JOINING THE STUDY?

At any stage you may change your mind and no longer participate. You can then stop participating and will not be punished in any way for this.

INSURANCE FOR THE STUDY PROVIDED BY THE MEDICAL RESEARCH COUNCIL

If you fall ill, suffer any side effects or if you are injured in any study related manner, contact the researchers immediately at the numbers provided below. The MRC, as sponsors of this study has taken out the necessary insurance to cover you as a research participant.

WHO CAN I ASK IF THERE ARE ANY PROBLEMS WITH THE STUDY?

Principal Investigator
Dr Naeemah Abrahams
Deputy Director
Gender & Health Research Unit, Medical Research Council
Cape Town Office
TEL: 021 9380823
FAX: 021 9380310
CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator
Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
Gender & Health Research Unit, Medical Research Council
RICE study- RK Khan Hospital, Chatsworth, Durban
TEL: 031 242 3721
Email: alesha.sewnath@mrc.ac.za
Study Office: Tel: 031 2423720

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Danie du Toit
Medical Research Council
P.O.Box 19070
Tygerberg
7505
Tel. 021-9380867
Email: adri.labuschagne@mrc.ac.za

MAIN STUDY SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I **DO AGREE** to join the study.

_____
Participant's Signature /Initial

OR

I **DO NOT AGREE** to join the study.

_____
Participant's Signature /Initial

_____	_____	_____
Participant's Signature	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

STORAGE OF LEFT OVER BLOOD SPECIMEN SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** for the left over blood specimens to be stored and used for future tests as discussed in this consent form**.**

_____
Participant Signature/Initial

OR

I **DO NOT AGREE** to have any of my left over blood specimens to be stored in the repository for use in studies in future.

_____
Participant Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

HAIR COLLECTION SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** to the collection of my hair sample as discussed in this consent form.

_____

Participant's Signature/Initial

OR

I **DO NOT AGREE** to give a hair sample for analysis in this study.

_____

Participant's Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

A STUDY ON WOMEN'S HEALTH AND WELL-BEING
USE OF BIOMETERIC SYSTEM
INFORMED CONSENT

RICE - WOMEN'S HEALTH AND WELLBEING STUDY

INTRODUCTION

You have decided to participate in the Women's Health and Well Being Study. In addition to the tests taken as part of the study, we are now asking you permission to take a fingerprint scans for our biometric systems. We have two systems the one is to ensure that there is no duplication of your results in our study and the second scan is to check if you are not participating in another study. It is important for us to know if you are with another study as it is not always safe to do so.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary, and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. Remember you are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the Medical Research Council of South Africa (MRC) Ethics Committee.

WHAT PROCEDURES WILL BE INVOLVED IN THIS RESEARCH?

We are asking you if we can do two scans of your fingerprints. This procedure is harmless and will not hurt you in any way. For the fingerprint scan, we will check to see if you are already on another study. The second fingerprint scan will be saved, and it will be linked to your unique identifier on the computer for the study. There will not be a picture of your thumbprint, but the computer will change the thumb print into a code. The code will then be printed into barcodes and this will be linked to your unique identifier number. These barcodes will then be placed on all your blood samples and your study file. In addition, whenever you return for follow-up visits, we will use the scanning of your thumb print to ensure we do not mix you up with someone else and also to make sure you are not with another study.

This is a longitudinal study, meaning that this study will be taking place over a long period of time, and sometimes studies that take place over a long period of time, patient samples and information gets misplaced. Using this system, we are making sure we link you to the samples without using your name. This is a precautionary measure that we will be taking in this study.

WHAT ABOUT CONFIDENTIALITY?

There will never be a picture of your thumb print. All fingerprints done for each participant will be stored as a code and under a unique identifier number. No one will know what your unique identifier is except the researchers working on the study.

WHAT IF I DO NOT WANT TO USE THE BIOMETRIC SYSTEM?

If you chose not to want to use the system, we will make an ID card for you for the study.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

Principal Investigator
Dr Naeemah Abrahams
Deputy Director, Gender & Health Research Unit, Medical Research Council
Cape Town: Office: 031 242 3688, CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator
Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
RICE study: RK Khan Hospital, Chatsworth, Durban
TEL: 031 242 3721
Email: alesha.sewnath@mrc.ac.za

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Moodley
Medical Research Council,
P.O.Box 19070, Tygerberg , 7505
Tel. 021-9380310
Email: adri.labuschagne@mrc.ac.za

USE OF BIOMETRIC SYSTEM SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I DO AGREEE to have my fingerprint used for the two biometric systems.

_____
Participant's Signature/Initial

OR

I DO NOT AGREE to have my fingerprint used for the two biometric systems.

_____
Participant's Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

A STUDY ON WOMEN'S HEALTH AND WELL-BEING

HIV INFORMED CONSENT
ADULT WOMEN

INTRODUCTION

You have decided to participate in the Women's Health and Well Being Study. As part of the study procedures, you will be asked to do an HIV Test. Like all the other tests that will be done in this study, there will be no names on any of the specimens, only a special study number.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. Remember you are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the Medical Research Council of South Africa (MRC) Ethics Committee.

WHAT ARE MY RIGHTS?

You have the following rights:

1. Not to be tested for the AIDS virus without your free and informed consent.
2. To be given all relevant information on the harms, risks and benefits of taking, or not taking, the HIV test.
3. To receive pre-test counselling which is private and confidential, and which will inform you more about the test and its implications before you give consent.
5. To have your test result treated confidentially
6. To receive post-test counselling

IS THE TEST ALWAYS CORRECT? CAN THERE BE MISTAKES?

Even though the tests are very accurate, if your test result shows that you may be infected with the AIDS virus, we will have this confirmed by doing some additional tests.

Sometimes, a false positive result may occur in a small number of cases. A false positive means that the test shows positive when the person is not infected with the virus, by doing further tests we can see if the test is really a positive. The clinic staff and the laboratories follow a strict procedure to prevent these potential mistakes. In order to minimize false positive results, two different tests are performed.

WHAT DOES IT MEAN IF THE TEST IS NEGATIVE?

If your test result is negative, it means that you are not currently infected, but it does not mean that you may not become infected in the future.

WHAT DOES IT MEAN IF THE TEST IS POSITIVE?

If your test result is positive, it means that you may be infected with the AIDS. However, to be sure, we will do an additional two tests to confirm the result. You will be called back to the research clinic and our research nurse will discuss the information with you so that you can understand clearly what the test result means. You will be given your CD4 and viral load and referred to your local clinic for further HIV management.

WHAT ABOUT TESTING AT HOME

When you struggle to attend the clinic, we will try and visit you at home to hear how we can assist you to come to the clinic. If you unable to attend the clinic we will ask you if we can do a finger prick HIV test at your home. We will follow all the confidential procedures as explained above.

WHAT ARE THE COSTS TO ME?

There is no direct cost to you for having an HIV test done.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

Principal Investigator
Dr Naeemah Abrahams
Deputy Director, Gender & Health Research Unit, Medical Research Council
Cape Town: Office: 031 242 3688, CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator
Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
RICE study: RK Khan Hospital, Chatsworth, Durban
TEL: 031 242 3721
Email: alesha.sewnath@mrc.ac.za

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Moodley
Medical Research Council,
P.O.Box 19070, Tygerberg , 7505
Tel. 021-9380310
Email: adri.labuschagne@mrc.ac.za

HIV TESTING SIGNATURE PAGE(Adult)

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I DO AGREEE to have a HIV test

_____
Participant (Signature/Initial)

OR

I DO NOT AGREE to have a HIV test.

_____
Participant (Signature/Initial)

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

A STUDY ON WOMEN'S HEALTH AND WELL-BEING

RESEARCH INVOLVING GENETIC STUDIES

INFORMED CONSENT

INTRODUCTION

You have decided to participate in the Women's Health and Wellbeing study**.** In addition to the tests taken as part of the study, we are now asking you permission to collect some of your blood for a study that involves DNA (Genetic) analysis.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the ethics Committee of the Medical Research Council of South Africa (MRC).

WHAT PROCEDURES WILL BE INVOLVED IN THIS RESEARCH?

A small blood sample (about 10-20 ml, equivalent to 1-2 tablespoons) will be taken from you by a trained nurse. Bloods will not be taken every time you visits us. The blood will be transported to a laboratory, where researchers will examine and identify your DNA from the blood sample. This sample will not be stored for later use but will be processed for immediate analysis and destroyed. The DNA sample will then help us to look for genetic differences and changes in your DNA. Findings these changes could tell us a lot about the development of stress-related mental health problems.

WHAT ABOUT CONFIDENTIALITY?

All blood samples collected will be identified only by a coded number and not your name to ensure to maintain confidentiality. The research records for this study will be kept in a secured area only accessible to the research team involved.

WHAT DOES THIS PARTICULAR RESEARCH STUDY INVOLVE?

In this study, we hope to be able to find genes that put a person at a higher risk for developing stress-related mental health problems, such as post-traumatic stress disorder (PTSD), depression and anxiety. We will do this by looking for changes in the genes and this will give us clues to the various factors that contribute to the development of these mental health problems.

WHAT IS DNA ANALYSIS OR GENETIC RESEARCH?

Genes are part of genetic material, also called DNA or RNA. Genes can be found by looking at a small blood sample and can be found in every cell our bodies. Our genes determine what we look like and

sometimes what kind of diseases we may be at risk in getting. Worldwide, researchers who do genetic research are continuously discovering new information that may be of great benefit to future generations and to people who suffer from different types of diseases or conditions. Our study will therefore assist in bringing about this new information to show how changes in our genes may affect our health and ability cope.

WHAT ARE THE RISKS TO ME IF I AGREE TO JOIN THIS STUDY?

- You may experience minor pain or bruising at the site where blood is taken. Occasionally, some people experience fleeing dizziness or feel faint when their blood is drawn.
- The samples collected will only be for this study and will not be shared with anyone.

WHAT ARE THE BENEFITS TO ME IF I DECIDE TO JOIN THIS STUDY

Your personal results will be made known to you **only if they indicate** that you may:-

- Have a definite risk for developing a particular disorder.
- Have a condition or predisposition to developing a condition that is treatable or avoidable e.g. by a lifestyle modification.

There are no direct benefits to you taking part in this study. However, the findings may benefit future patients with stress-related mental health problems. This new information will provide us with a better understanding of the development of stress-related mental health problems and may result in the development of ways to lower the risk for these disorders, as well as helping us find new treatments.

WHAT ARE THE COSTS TO ME?

As explained earlier you will not be paid to take part in this study although you will be given R80 at each of the interviews to compensate for time spent completing the questionnaire and providing samples. You will also be given money to cover travel costs for each of the visits and we will provide you with a snack every time you come for the interviews.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

Principal Investigator:

Principal Investigator
Dr Naeemah Abrahams
Deputy Director, Gender & Health Research Unit, Medical Research Council
Cape Town: Office: 031 242 3688, CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator

Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
RICE study: RK Khan Hospital, Chatsworth, Durban
TEL: 031 242 3721

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee

Prof. Danie du Toit

Medical Research Council

P.O.Box 19070, Tygerberg, 7505

Tel. 021-9380687

Email: adri.labuschagne@mrc.ac.za

GENETIC STUDIES SIGNATURE PAGE (adult)

I declare that: -

• I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

• I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **AGREE** to take part in a genetic research study entitled

_____

Participant Signature/Initial

OR

I **DO NOT AGREE** to take part in a genetic research study

_____

Participant Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

ADDENDUM D

Demographic and clinical characteristics of the rape impact cohort evaluation (RICE) group compared to the data subsets used in this study

In Chapter 3 of this dissertation (risk and protective factors affecting the symptom trajectory of posttraumatic stress disorder post-rape) we present the findings from the RICE cohort with the exclusion of participants with posttraumatic stress disorder (PTSD) at baseline, due to a trauma other than the rape. When comparing the Chapter 3 subset to the entire RICE cohort, we found a number of significant differences in baseline demographic and clinical characteristics. The participants included in the subset had a slightly lower score on childhood trauma (mean difference = 0.04), social support (mean difference = 0.05), perceived stress (mean difference = 0.05), rape stigma (mean difference = 0.06) and PTSD score (mean difference = 4.4). Al of the aforementioned variables were measure on a Likert scale and given the small differences observed it is unlikely that they will be of clinical significance. The larger difference in PTSD scores may be of clinical significance and is likely the result of excluding participants with PTSD at baseline (due to an event other than the rape) from the Chapter 3 subset. The findings of the comparisons between the RICE cohort and the Chapter 3 subset is presented in Table 1.

In Chapter 4 of this dissertation (genome-wide differentially methylated genes associated with posttraumatic stress disorder and longitudinal change in methylation in rape survivors) we present the findings from a case-control subgroup (discovery/validation subset) selected by matching consecutive cases of PTSD to controls, based on baseline demographic and clinical characteristics. We also included a replication set selected based on the quality and availability of DNA samples (replication subset). Participants included in the discovery/validation subset were less likely to use chronic medication at baseline (mean difference = 2.7%) and showed decrease alcohol consumption at baseline (mean difference = 0.04) compared to the entire RICE cohort (see Table 2). There were no significant differences in demographic and clinical characteristics between the replication sample and the entire RICE cohort (see Table 3).

In Chapter 4 and 5 (*FKBP5* intron 7 methylation and the trajectory of PTSD symptoms in rape-exposed women) of this dissertation we present the findings from the combined discovery/validation and replication subsets. We found that participants included in the combined subset showed decreased alcohol consumption (mean difference = 0.07) at

baseline compared to the participants include in the entire RICE cohort. No other differences were observed (see Table 4).

Table 1: *Baseline demographic and clinical characteristics of the RICE cohort compared to the chapter 3 subset*

	RICE cohort set (n=852)		Chapter 3 subset (n=639)		Comparison of RICE cohort to chapter 3 subset		
	n (%)	*M (SD)*	n (%)	*M (SD)*	χ^2	*z*	*p*
Age[1]	852(100)	25.0(5.3)	639(100)	24.7(5.3)		-0.95	.344
Secondary education completed[2]	484(56.8)		368(57.6)		0.09		.763
Employed[2]	185(21.7)		138(21.6)		0.00		.957
In a relationship/married[2]	664(78.0)		512(80.1)		0.91		.340
BMI[1]	852(100)	26.0(6.3)	639(100)	25.9(5.3)		-0.02	.988
Smoker[2]	125(14.7)		88(13.8)		0.25		.617
HIV positive[2]	411(48.2)		297(46.5)		0.34		.558
Chronic medication use[2,3]	475(55.8)		353(55.2)		0.04		.851
Childhood trauma score[1]	852(100)	16.7(3.9)	639(100)	16.3(3.5)		-1.98	.047*
Number of lifetime traumas[1,3]	852(100)	1.5(1.5)	639(100)	1.4(1.4)		-1.22	.222
Resilience score[1]	850(100)	50.8(6.4)	638(100)	50.5(6.3)		-0.76	.447
Social support score[1]	850(100)	25.5(5.1)	638(100)	25.0(5.0)		-6.98	.000*
Perceived stress score[1]	850(100)	24.1(4.9)	638(100)	23.6(4.6)		-9.70	.000*
Rape stigma score[1]	850(100)	21.3(7.0)	638(100)	20.7(7.0)		-33.07	.000*
Alcohol use severity[1]	852(100)	2.1(2.5)	639(100)	2.1(2.5)		-0.76	.446
Depression symptom score[1]	852(100)	34.0(12.6)	639(100)	33.0(12.6)		-1.46	.146
PTSD symptom score[1]	852(100)	71.9(31.7)	639(100)	67.5(31.0)		-2.18	.029*

[1]Continous variables; [2]categorical variables; [3]lifetime traumas refer to directly experiencing the trauma; *p < .05

Abbreviations: Rape impact cohort study (RICE); mean (M); standard deviation (SD); body mass index (BMI); posttraumatic stress disorder (PTSD).

Table 2: *Baseline demographic and clinical characteristics of the RICE cohort compared to the chapter 4 discovery/validation subset*

	RICE cohort set (n=852)		Chapter 4 discovery/validation set (n=47)		Comparison of RICE cohort to chapter 4 discovery/validation set		
	n (%)	*M (SD)*	n (%)	*M (SD)*	χ^2	*z*	*p*
Age[1]	852(100)	25.0(5.3)	96(100)	25.2(5.4)		-1.23	.218
Secondary education completed[2]	484(56.8)		57(59.4)		0.23		.630
Employed[2]	185(21.7)		22(22.9)		0.92		.338
In a relationship/married[2]	664(78.0)		76(79.2)		0.02		.896
BMI[1]	852(100)	26.0(6.3)	96(100)	25.9(6.1)		-0.48	.633
Smoker[2]	125(14.7)		12(12.5)		0.59		.442
HIV positive[2]	411(48.2)		46(47.9)		2.02		.155
Chronic medication use[2,3]	475(55.8)		51(53.1)		4.26		.039*
Childhood trauma score[1]	852(100)	16.7(3.9)	96(100)	16.7(3.4)		-0.46	.644
Number of lifetime traumas[1,3]	852(100)	1.5(1.5)	96(100)	1.4(1.4)		-0.67	.501
Resilience score[1]	850(100)	50.8(6.4)	96(100)	49.4(7.5)		-1.52	.129
Social support score[1]	850(100)	25.5(5.1)	96(100)	24.6(5.1)		-1.17	.243
Perceived stress score[1]	850(100)	24.1(4.9)	96(100)	23.7(4.3)		-0.98	.330
Rape stigma score[1]	850(100)	21.3(7.0)	96(100)	20.9(7.2)		-0.74	.462
Alcohol use severity[1]	852(100)	2.1(2.5)	96(100)	1.7(2.4)		-2.21	.027*
Depression symptom score[1]	852(100)	34.0(12.6)	96(100)	32.0(13.0)		-0.68	.495
PTSD symptom score[1]	852(100)	71.9(31.7)	96(100)	66.0(32.4)		-1.30	.195

[1]Continous variables; [2]categorical variables; [3]lifetime traumas refer to directly experiencing the trauma; *p < .05

Abbreviations: Rape impact cohort study (RICE); mean (M); standard deviation (SD); body mass index (BMI); posttraumatic stress disorder (PTSD).

Table 3: *Baseline demographic and clinical characteristics of the RICE cohort compared to the chapter 4 replication subset*

	RICE cohort (n=852)		Chapter 4 replication set (n=49)		Comparison of RICE cohort to chapter 4 replication set		
	n (%)	*M (SD)*	n (%)	*M (SD)*	χ^2	*z*	*p*
Age[1]	852(100)	25.0(5.3)	49(100)	24.6(5.5)		-0.75	.454
Secondary education completed[2]	484(56.8)		25(51)		0.71		.400
Employed[2]	185(21.7)		9(18.4)		0.34		.560
In a relationship/married[2]	664(78.0)		38(77.6)		0.01		.934
BMI[1]	852(100)	26.0(6.3)	49(100)	25.8(5.7)		-0.25	.801
Smoker[2]	125(14.7)		7(14.3)		0.01		.935
HIV positive[2]	411(48.2)		19(38.8)		1.87		.172
Chronic medication use[2,3]	475(55.8)		32(65.3)		1.90		.168
Childhood trauma score[1]	852(100)	16.7(3.9)	49(100)	16.2(2.5)		-0.62	.534
Number of lifetime traumas[1,3]	852(100)	1.5(1.5)	49(100)	1.1(1.2)		-1.66	.098
Resilience score[1]	850(100)	50.8(6.4)	49(100)	50.1(6.9)		-0.03	.980
Social support score[1]	850(100)	25.5(5.1)	49(100)	24.6(4.7)		-1.14	.253
Perceived stress score[1]	850(100)	24.1(4.9)	49(100)	24.2(3.7)		-0.19	.850
Rape stigma score[1]	850(100)	21.3(7.0)	49(100)	21.4(7.1)		-0.01	.994
Alcohol use severity[1]	852(100)	2.1(2.5)	49(100)	1.9(2.5)		-0.67	.501
Depression symptom score[1]	852(100)	34.0(12.6)	49(100)	31.6(12.1)		-1.26	.208
PTSD symptom score[1]	852(100)	71.9(31.7)	49(100)	67.1(30.3)		-1.20	.230

[1]Continous variables; [2]categorical variables; [3]lifetime traumas refer to directly experiencing the trauma; *p < .05

Abbreviations: Rape impact cohort study (RICE); mean (M); standard deviation (SD); body mass index (BMI); posttraumatic stress disorder (PTSD).

Table 4: *Baseline demographic and clinical characteristics of the RICE cohort compared to the chapter 4 and 5 combined subset*

	RICE cohort set (n=852)		Chapter 4&5 combined set (n=96)		Comparison of RICE cohort to chapter 4&5 combined set		
	n (%)	*M (SD)*	n (%)	*M (SD)*	χ^2	*z*	*p*
Age[1]	852(100)	25.0(5.3)	47(100)	25.9(5.4)		-0.32	.750
Secondary education completed[2]	484(56.8)		32(68.1)		0.23		.630
Employed[2]	185(21.7)		13(27.7)		0.07		.787
In a relationship/married[2]	664(78.0)		38(80.9)		0.07		.798
BMI[1]	852(100)	26.0(6.3)	47(100)	26.0(6.5)		-0.50	.616
Smoker[2]	125(14.7)		5(10.6)		0.33		.563
HIV positive[2]	411(48.2)		27(57.4)		0.00		.952
Chronic medication use[2,3]	475(55.8)		19(40.4)		0.25		.615
Childhood trauma score[1]	852(100)	16.7(3.9)	47(100)	17.2(4.1)		-0.12	.906
Number of lifetime traumas[1,3]	852(100)	1.5(1.5)	47(100)	1.6(1.5)		-0.69	.488
Resilience score[1]	850(100)	50.8(6.4)	47(100)	48.8(8.2)		-1.09	.278
Social support score[1]	850(100)	25.5(5.1)	47(100)	24.7(5.5)		-1.64	.101
Perceived stress score[1]	850(100)	24.1(4.9)	47(100)	23.2(4.9)		-0.36	.716
Rape stigma score[1]	850(100)	21.3(7.0)	47(100)	20.3(7.3)		-0.27	.785
Alcohol use severity[1]	852(100)	2.1(2.5)	47(100)	1.4(2.2)		-2.21	.027*
Depression symptom score[1]	852(100)	34.0(12.6)	47(100)	32.4(13.9)		-1.37	.172
PTSD symptom score[1]	852(100)	71.9(31.7)	47(100)	64.9(34.7)		-1.59	.112

[1]Continous variables; [2]categorical variables; [3]lifetime traumas refer to directly experiencing the trauma; *p < .05

Abbreviations: Rape impact cohort study (RICE); mean (M); standard deviation (SD); body mass index (BMI); posttraumatic stress disorder (PTSD).

ADDENDUM E

Permission to include the published article presented in Chapter 2 in this dissertation